09/623514
5640

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

RECD 27 MAR 2001

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
43922-PT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/01202	16/12/1999	17/12/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/54		
Applicant		
NATIONAL RESEARCH COUNCIL OF CANADA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14/07/2000	21.03.2001
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Montero Lopez, B Telephone No. +31 70 340 3739

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01202

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1,3-5,7-42, 44-46	as originally filed			
2,2bis,6,43,47	as received on	14/12/2000	with letter of	14/12/2000

Claims, No.:

1-23	as received on	14/12/2000	with letter of	14/12/2000

Drawings, No.:

1-13	as originally filed

Sequence listing part of the description, pages:

1-20, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-13, 17-21, 23
	No:	Claims	1, 2, 14-16, 22,
Inventive step (IS)	Yes:	Claims	5, 23
	No:	Claims	1-4, 6-22
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the

claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: R61u012 Database Entry Ac005917; Accession number AC005917; 4 November 1998; LIN X. ET AL.:"Arabidopsis thaliana chromosome II section 113 of 255 of the complete sequence"
D2: VESNA KATAVIC ET AL.:"Alteration of seed fatty acid composition by an ethyl methanesulfonate-induced mutation in Arabidopsis thaliana affecting diacylglycerol acyltransferase activity" PLANT PHYSIOLOGY, vol. 108, 1995, pages 399-409

1. Claims 1, 2 and 22 encompass a plant DNA molecule which includes, respectively, the whole or part of SEQ ID NO:1 and 3 or a sequence substantially homologous thereto.

1.1. Document D1 discloses a Arabidopsis thaliana DNA sequence identical to nucleotides 1-528 of SEQ ID NO: 1 in nucleotides 22710-23238 and to the whole SEQ ID NO:3 in nucleotides 21090-26283. This sequence is identified as encoding a diacylglycerol O-acyltransferase (see page 5 of D1). Document D1 constitutes an update of an original record submitted on 03.11.1998, subsequently updated on 19.11.1998 and 21.11.1998. These records provide corrections to the originally submitted DNA sequence. All these sequences submitted before the priority dated of the application show a high degree of homology in the fragment corresponding to nucleotides 21090-26283 with SEQ ID NOs:1 and 3. They are considered therefore to be substantially homologous thereto. Claims 1, 2 and 22 are therefore not novel and do not meet the requirements of Article 33(3) PCT.

2. Claims 3, 4, and 6-13 relate to the introduction of the DNA molecule into a vector in a sense or antisense orientation, and into plant seeds and plants. Document D1 does not disclose these particular embodiments and therefore claims 3, 4, and 6-13 are novel and comply with the requirements of Article 33(2) PCT.

2.1. The introduction of the Arabidopsis thaliana sequence disclosed in D1 into

vectors, plant seeds and plants constitute a routine obvious application which the skilled person would put into practice using standard techniques in the art without the need of exercising any inventive skill. Consequently, claims 3, 4, and 6, 7 and 9-13 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

2.2. Claim 8 is directed to plasmid pDGATcDNA (ATCC PTA-989). This plasmid contains the cDNA sequence disclosed in SEQ ID NO:3. Since document D1 discloses this sequence, with the exception of slight sequencing errors, the skilled person would have no difficulty in devising a plasmid equivalent to the plasmid claim in claim 8. Consequently, claim 8 lacks inventive step and does not comply with the requirements of Article 33(3) PCT.

3. Claim 5 is directed to a vector containing SEQ ID NO:23. No such sequence has been disclosed in the state of the art and therefore claim 5 is novel according to Article 33(2) PCT.

3.1. Moreover, sequence SEQ ID NO:23 is constituted by SEQ ID NO:1, containing an insertion of 81 nucleotides, for which no hint exists in the state of the art, and which the skilled person would not have been able to devise without exercising an inventive step. Claim 5, therefore, involves an inventive step according to Article 33(3) PCT.

4. Claims 14-18 relate to plant seeds and plants containing a homologous sequence to SEQ ID NO:1 an exhibiting the altered properties as described in claims 14-18.

4.1. Document D2, which is considered to represent the most relevant state of the art, discloses (cf. abstract, page 399, col. right, par. last - page 400, col. left, par. 1) an Arabidopsis thaliana mutant, designated AS11, which shows a reduced diacylglycerol acyltransferase activity. It is clear that the AS11 mutant disclosed in D2 contains a mutated sequence encoding diacylglycerol acyltransferase of SEQ ID NO:1; the specification in claim 11 that it consists of a recombinant sequence constitutes an attempt to define a product by its method of preparation. A particular nucleotide sequence remains the same regardless of its method of preparation. In addition, the seeds and plants disclosed in D2 show altered seed

oil content, altered diacylglycerol content and altered fatty acyl composition (see page 402, col. left, par. 2 - col. right, par. 2 and page 403, col. left, par. 2 - page 407, col. left, par. 1). Consequently, claims 14-16 are not novel and do not meet the requirements of Article 33(2) PCT.

4.2. Claims 17 and 18 refer to plants and seeds exhibiting an enhanced biomass. Document D2 is silent about this property and therefore, claims 17 and 18 are considered to be novel and comply with the requirements of Article 33(2) PCT. However, it is considered that an enhanced biomass is an inevitable consequence of the alterations in seed oil content of the AS11 mutants. Consequently, claims 17 and 18 are considered not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

5. Claims 19-21 refer to a method of producing transgenic plants by introducing into the genome of said plant a nucleotide sequence comprising SEQ ID NOs. 1 or 3 or a sequence substantially homologous thereto. Since document D1 does not disclose such an embodiment, claims 19-21 are novel and fulfill the requirements of Article 33(2) PCT.

5.1. However, the introduction of the nucleotide sequence disclosed in D1 into a plant and synthesis of a transgenic plant would be an obvious application for the skilled person which he/she would put into practice without the need of exercising any inventive step. Consequently, claims 19-21 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

6. Claim 23 refers to a method of changing the oil content, acyl composition or diacylglycerol/triacylglycerol proportions of seed oils by transforming a plant seed with SEQ ID NO:1, 3 or 23 or a part or homologous thereof. Since document D1 does not disclose such a method, claim 23 is novel and fulfills the requirements of Article 33(2) PCT.

6.1. Sequence SEQ ID NO:3 has already been described in D1; however, its identification as encoding a diacylglycerol acyltransferase was only made after the priority date. Therefore, it would not have been obvious for the skilled person that the oil content of plant seeds can be altered by transforming them with the

sequence disclosed in D1. Consequently, claim 23 involves an inventive step and meets the requirements of Article 33(3) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
PCT/US98/17883	29/12/1999	28/08/1998	24/06/1998
PCT/US99/15243	13/01/2000	10/06/1999	02/07/1998

Re Item VIII
Certain observations on the international application

1. Claims 1 and 2 refer to "isolated" and "purified" DNA. The degree of isolation or purification is, however, not a technical feature of a preparation and the terms "isolated" and "purified" are therefore disregarded (Article 6 PCT).

2. The relative term "substantially" used in claims 1-4, 10, 11, 19, 22 and 23 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

3. The term "homologous" used in claim 1-4, 10, 11, 19, 22 and 23 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

6. Claims 10-13 attempt to define a product, a plant or plant seed, according to the

process to obtain it, by introduction or transformation thereof with a recombinant nucleotide sequence. However, the method of preparation does not impart any limitation to the product . A claim directed to a product according to the process to obtain the same is therefore construed as a claim to the product as such. Claims 10-13 are considered, therefore, to encompass plants or plant seeds containing the concerned sequence irrespective of the process for their preparation (Article 6 PCT).

DGAT from cotyledons of germinating soybean seeds was reported (Kwanyuan and Wilson, 1986), detailed molecular characterization of this enzyme is lacking.

Reference is made to R61u012 Database Entry Ac005917; Accession number AC005917; 4 November 1998; LIN X. ET AL : "Arabidopsis thaliana chromosome II section 113 of 255 of the complete sequence". This relates to a nucleotide sequence deposit first submitted to the NCBI GenBank on November 3, 1998 without any identification of putative coding sequences contained therein. There was no reference in the deposited materials to a "diacylglycerol O-acyltransferase gene".

Reference is also made to VESNA KATAVIC ET AL.: "Alteration of Seed Fatty Acid Composition by an Ethyl Methanesulfonate-induced Mutation in Arabidopisis thaliana Affecting Diacylglycerol Acyltransferase Activity"; PLANT PHYSIOLOGY, vol. 108, 1995, pp. 399-409. This reference discloses an Arabidopsis mutant designated AS11 that has reduced diacylglycerol acyltransferase activity. The reference does not disclose any DNA sequences whatsoever and teaches only that alterations in DGAT activity may lead to changes in fatty acid content.

Accordingly, while the Kennedy pathway is known and shows the steps in the biosynthesis of TAGs in plants, there has not been any identification and use of a genetic element that can be used reliably in plants to modify TAG synthesis and composition in a way that may be exploited commercially.

DISCLOSURE OF THE INVENTION

An object of the invention is to identify, isolate and clone a genetic element that may be used to modify the natural formation of triacylglycerols in plants in order to increase the yield of commercial plant oils, or to modify their composition to achieve specific commercial improvements of plants and plant products.

Another object of the invention is to identify, isolate and characterize diacylglycerol acyltransferase (DGAT) gene and cDNA sequences from Arabidopsis and to utilize these sequences in the genetic manipulation of plants.

Another object of the invention is to provide a vector containing the full-length DGAT cDNA sequence from *Arabidopsis* in a sense orientation under the control of a seed-specific promoter (e.g. napin; See Josefsson et al., 1987; Radke et al., 1988; Voelker et al., 1992), for re-introducing into *Arabidopsis* or for introducing into other plants.

Another object of the invention is to provide a vector containing a genomic fragment from *Arabidopsis* consisting of the full-length DGAT gene under the control of its own 5' upstream regulatory sequences, for re-introducing into *Arabidopsis* or for introducing into other plants.

Another object of the invention is to provide a method to construct a vector containing the full-length DGAT sequence or a significant portion of the DGAT sequence from *Arabidopsis*, in an antisense orientation under control of either a constitutive or a seed-specific promoter, for re-introducing into *Arabidopsis* or for introducing into other plants.

expression of a full-length cDNA in yeast; (3) the characterization and isolation of the DGAT (*TAG1*) gene from Arabidopsis mutant AS11 containing an insertion mutation in the DGAT gene and a seed oil phenotype which consists of an altered DAG/TAG ratio, and an altered oil content and acyl composition; (4) complementation of the AS11 mutant by insertion of the DGAT cDNA sequence to restore a wild-type fatty acid composition; (5) the over-expression of the DGAT cDNA in wild-type *A. thaliana* transgenic plants which produce seeds with an increased oil content, increased average seed weight and altered seed oil acyl composition.

The *A. thaliana* DGAT structure is significantly homologous (over 40% identical over a region of more than 400 amino acids) to its mammalian counterparts, and is highly homologous to subsequently reported putative *B. napus* DGATs at both the nucleotide (92%) and the deduced amino acid (90%) levels (Nykiforuk et al, 1999; GenBank/EMBL Accession No AF155224; AF164434).

The DGAT of the current invention is useful in manipulating DGAT activity, and triacylglycerol bioassembly in plants. For example, by transforming plants with a construct containing the DGAT gene in a sense orientation, under the control of a tissue-specific promoter (e.g. seed-specific promoter napin), the expression of DGAT and accumulation of seed oil can be enhanced or the acyl composition of the seed oil altered. Yet another example would be to express the DGAT cDNA under the control of a constitutive promoter (e.g. 35S (Datla et al., 1993)) to increase the TAG content of vegetative tissues (leaves, roots, stems). This may have particular advantages for altering the starch/oil ratio in root crops.

Alternatively, DGAT expression can be silenced to some degree by anti-sense or co-suppression (Transwitch) phenomena (De Lange et al., 1995; Mol et al., 1990; Jorgensen and Napoli, 1994; Kinney, 1995; Vaucheret et al, 1998; Taylor, 1998). For example, silencing DGAT in a seed specific manner may result in a reduction in TAG accumulation. This could have applications in reducing the oil content in seed barley to enhance stability during storage. As a second example, seed-specific silencing may lead to a relatively high accumulation of DAG or an increase in the DAG/TAG ratio in the developing or mature seed. As yet another example, the expression of the mutated DGAT gene which results in a 27 amino

Mogami, K., O'Donnell, P.T., Bernstein, S.I., Wright, T.R.F and Emerson, C.P., JR. (1986) Mutations of the Drosophila myosin heavy-chain gene: effects on transcription, myosin accumulation, and muscle function. Proc. Nat'l. Acad. Sci. USA. 83, 1393-1397.

Mol, J.M.N., Van der Krol, A.R., Van Tunen, A.J., Van Blokland, R., De Lange, P. and Stuitje, A.R. (1990) Regulation of plant gene expression by antisense RNA. FEBS Lett. 268: 427-430.

Moloney, M.M., Walker, J.M. and Sharma, K.K. (1989) High efficiency transformation of Brassica napus using Agrobacterium vectors. Plant Cell Rep. 8: 238-242.

Nagiec, M.M., Wells, G.B., Lester, R.L., and Dickson, R.C. (1993). A suppressor gene that enables Saccharomyces cerevisiae to grow without making sphingolipids encodes a protein that resembles an Escherichia coli fatty acyltransferase. J. Biol. Chem. 268, 22156-22163.

Nehra, N.S., Chibbar, R.N., Leung, N., Caswell, K., Mallard, C., Steinhauer, L. Baga, M. and Kartha K.K. (1994) Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. Plant J 5: 285-297.

Nykiforuk C, Laroche A and Weselake RJ (1999) Isolation and sequence analysis of a novel cDNA encoding a putative diacylglycerol acyltransferase from a microspore-derived cell suspension culture of Brassica napus L. cv Jet Neuf (Accession No. AF155224).), Plant Physiology 120: 1207.

Oelkers, P., Behar, A., Cromley, D., Billheimer, J.T. and Sturley, S.T. (1998) Characterization of two human genes encoding acyl Coenzyme A: cholesterol acyltransferase-related enzymes. J. Biol. Chem. 273, 26765-26771.

Okagaki, R.J., Neuffer, M.G. and Wessler, S.R. (1991) A deletion common to two independently derived Waxy mutations in maize. Genetics 128, 425-431.

Okuley, J., Lightner, J., Feldmann, K., Yadav, N., Lark, E. and Browse, J. (1994) Arabidopsis fad2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. The Plant Cell 6: 147-158.

Weselake, R.J., Taylor, DC, Pomeroy, M.K., Lawson SL, and Underhill EW (1991) properties of diacylglycerol acyltransferase from microspore-derived embryos of Brassica napus L. Phtochemistry : 30: 3533-3538.

Weselake, R.J., Pomeroy, M.K., Furukawa, T.L., Golden, J.L., Little, D.B. and Laroche, A. (1993) Developmental profile of diacylglycerol acyltransferase in maturing seeds of oilseed rape and safflower and micro-spore-derived cultures of oilseed rape. Plant Physiol. 102, 565-571.

Wilson, R.F. and Kwanyuan P. (1986) Triacylglycerol synthesis and metabolism in germinating soybean cotyledons. Biochim. Biophys. Acta 877, 231-237.

Yang, H., Bard, M., Bruner, D.A., Gleeson, A., Deckelbaum, R.J., Aljinovic, G., Pohl, T.M., Rothstein, R. and Sturley, S.L. (1997) Sterol esterification in yeast: a two-gene process. Science 272, 1353-1356.

Yu, C., Kennedy, N.J., Chang, C.C.Y. and Rothblatt, J.A. (1996) Molecular cloning and characterization of two isoforms of Saccharomyces cerevisiae Acyl-CoA: Sterol Acyltransferase. J. Biol. Chem. 271, 24157-24163.

Zou, J-T., Katavic. V., Giblin, E.M., Barton, D.L., MacKenzie, S.L., Keller, W.A., Hu, X. and Taylor, D.C. (1997) Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. The Plant Cell 9: 909-923.

CLAIMS:

1. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:1 or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

2. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:3 or a part of SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:3.

3. A vector that contains a nucleic acid sequence according to SEQ ID NO:1, or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

4. A vector that contains a nucleic acid sequence according to SEQ ID NO:3, or a part of SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:3.

5. A vector that contains a deoxyribonucleic acid sequence according to SEQ ID NO:23.

6. A vector according to claim 3 or claim 5, characterized in that said sequence is present in said vector in a sense orientation.

7. A vector according to claim 3, characterized in that said sequence is present in said vector in an anti-sense orientation.

8. Plasmid pDGATcDNA (ATCC PTA-989).

9. Plasmid pDGATgene (ATCC PTA-988).

10. A plant having a genome, characterized in that the genome contains an introduced recombinant nucleotide sequence of SEQ ID NO:1, or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

11. A plant seed having a genome, characterized in that said genome contains an introduced recombinant nucleotide sequence of SEQ ID NO:1, or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

12. A genetically transformed plant, characterized in that said plant has a genome that has been transformed by a vector according to claim 3 or claim 4 or claim 5.

13. A genetically transformed plant seed, characterized in that said seed has been transformed by a vector according to claim 3 or claim 4 or claim 5.

14. A plant seed as claimed in Claim 11 or Claiim 13, characterized by exhibiting an altered seed oil content compared to the seed oil content of seeds of genomically-unmodified plants of the same genotype grown in identical conditions at the same time.

15. A plant seed as claimed in Claim 11 or Claim13, characterized by exhibiting an altered diacylglycerol content in its seed oil compared to the diacylglycerol content of seeds of genomically-unmodified plants of the same genotype grown in identical conditions at the same time.

16. A plant seed as claimed in 11 or 13, characterized by exhibiting a seed oil with an altered fatty acyl composition compared to the fatty acid composition of plants of the same genotype grown in identical conditions at the same time.

17. A plant as claimed in 10 or 12, characterized by exhibiting an enhanced biomass compared to the biomass of genomically-unmodrfied plants of the same genotype grown in identical conditions at the same time.

18. A seed as claimed in 11 or 13, characterized by exhibiting an enhanced biomass compared to the biomass of genomically-unmodified plants of the same genotype grown under identical conditions at the same time.

19. A method of producing transgenic plants by introducing a recombinant nucleotide sequence into a genome of said plant, characterized in that said nucleotide sequence introduced into said genome includes SEQ ID NO:1, SEQ ID NO.3 or SEQ ID NO:23; or a part of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:23; or a sequence that is substantially homologous to SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:23.

20. A method according to claim 19, characterized in that said plant is a member of the Brassicaceae.

21. A method according to claim 19, characterized in that said plant is selected from the group consisting of Arabidopsis thaliana, borage (*Borago* spp.), Canola, castor (*Ricinus communis*), cocoa bean (*Theobroma cacao*), corn (*Zea mays*), cotton (*Gossypium* spp), *Crambe* spp., *Cuphea* spp., flax (*Linum* spp.), *Lesquerella* and *Limnanthes* spp., Linola, nasturtium (*Tropaeolum* spp.), *Oenothera* spp., olive (*Olea* spp.), palm (*Elaeis* spp.), peanut (*Arachis* spp.), rapeseed, safflower (*Carthamus* spp.), soybean (*Glycine* and *Soja* spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.), *Vernonia* spp., wheat (*Triticum* spp.), barley (*Hordeum* spp.), rice (*Oryza* spp.), oat (*Avena* spp.) sorghum (*Sorghum* spp.), rye (*Secale* spp.) and other members of the *Gramineae*.

22. An isolated plant DNA sequence or part thereof, characterized in that the sequence is substantially homologous to at least a part of SEQ ID NO:1 or SEQ ID NO:3.

23. A method of changing the oil content, acyl composition or diacylglycerol/ triacylglycerol proportions of the seed oil of plant seeds by introducing a sense or anti-sense recombinant nucleic acid construct into a plant transformation vector, using the vector to transform the genome of a plant or plant seed, and then growing the plant or plant seed and extracting the oil from the plant seed, characterized in that said recombinant nucleic acid sequence comprises SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:23, or a part of SEQ ID NO.1, SEQ ID NO:3 or SEQ ID NO:23; or a sequence that is substantially homologous to SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:23.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
43922-PT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 99/ 01202	16/12/1999	17/12/1998
Applicant		
NATIONAL RESEARCH COUNCIL OF CANADA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/01202

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/54 C12N15/82 A01H5/00 A01H5/10 A01H3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R61u012 Database Entry Ac005917 Accession number AC005917; 4 November 1998 LIN X. ET AL.:"Arabidopsis thaliana chromosome II section 113 of 255 of the complete sequence" XP002133608 nucleotides 21090-23400 -& LIN X. ET AL. : "Sequence and analysis of chromosome II of Arabidopsis thaliana" NATURE, vol. 402, 1999, pages 761-768, XP000877287 LONDON GB --- -/--	1-4,6-8, 10-13, 19-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2000	14/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

6

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CASES S ET AL: "Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 95, no. 22, 27 October 1998 (1998-10-27), pages 13018-13023, XP002122745 ISSN: 0027-8424 cited in the application the whole document ---	1,2,22
X	VESNA KATAVIC ET AL.: "Alteration of seed fatty acid composition by an ethyl methanesulfonate-induced mutation in Arabidopsis thaliana affecting diacylglycerol acyltransferase activity" PLANT PHYSIOLOGY, vol. 108, 1995, pages 399-409, XP002915657 cited in the application page 399, right-hand column, last paragraph -page 400, left-hand column, paragraph 1 page 402, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 403, right-hand column, last paragraph -left-hand column, paragraph 1 page 405, right-hand column, last paragraph -page 408, left-hand column, paragraph 1 ---	10-18
E	WO 99 67403 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 29 December 1999 (1999-12-29) page 3, line 27 -page 6, line 5 page 10, line 2 - line 25 page 14, line 16 - line 21 page 15, line 24 -page 16, line 28 page 33, line 4 - line 32 page 39, line 28 -page 40, line 11 ---	1-4,6, 10,12, 19-23
E	WO 00 01713 A (CALGENE LLC) 13 January 2000 (2000-01-13) page 4, line 10 - line 17 page 4, line 20 -page 5, line 2 page 11, line 27 -page 24, line 21; examples 11-14,16 --- -/--	1-4,6, 10,12, 19,22,23

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Empln Database Entry Ath238008 Accession number AJ238008; 18 June 1999 ZOU J. ET AL.:"The Arabidopsis thaliana TAG1 gene encodes for a diacylglycerol acyltransferase" XP002133609 cited in the application the whole document ---	1,2,22
P,X	ZOU, JITAO ET AL: "The Arabidopsis thaliana TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene" PLANT J. (1999), 19(6), 645-653 , XP002133607 the whole document ---	1,2,22
P,X	EMBL Database Entry AF155224 Accession number AF155224; 30 June 1999 NYKIFORUK C.L. ET AL.:"Brassica napus putative diacylglycerol cyltransferase (DGAT2) mRNA" XP002133639 cited in the application the whole document & NYKIFORUK C.L. ET AL.: "Isolation and sequence analysis of a novel cDNA encoding a putative diacylglycerol acyltransferase from a microspore-derived cell suspension culture of Brassica napus L. cv Jet Neuf (Accession No. AF155224) (PGR99-123)." PLANT PHYSIOLOGY, vol. 120, no. 4, 1999, pages 1207-1207, LONDON GB ---	1,2,22
P,X	EMBL Database Entry AF164434 Accession number AF164434; 26 July 1999 NYKIFORUK C.L. ET AL.:"Brassica napus putative diacylglycerol acyltransferase (DGAT1) mRNA" XP002133640 cited in the application the whole document & NYKIFORUK C.L. ET AL.: "Isolation and characterization of a cDNA encoding a second putative diacylglycerol acyltransferase from a microspore-derived cell suspension culture of Brassica napus L. cv Jet Neuf (Accession No. AF164434) (PGR99-158)" PLANT PHYSIOLOGY, vol. 121, no. 3, 1999, pages 1053-1053, --- -/--	1,2,22

6

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	HOBBS D H ET AL: "Cloning of a cDNA encoding diacylglycerol acyltransferase from Arabidopsis thaliana and its functional expression" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 452, no. 3, 11 June 1999 (1999-06-11), pages 145-149, XP002122747 ISSN: 0014-5793 the whole document	1,2,22
P,X	WO 99 63096 A (CALGENE LLC) 9 December 1999 (1999-12-09) page 3, line 24 -page 4, line 17 page 6, line 13 -page 8, line 16 page 12, line 30 -page 20, line 10; examples 1-4,7,8 sequence listing SEQ ID NO:1	1-4,6, 10-13, 19,22,23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/CA 99/01202

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9967403 A	29-12-1999	WO 9967268 A	29-12-1999
WO 0001713 A	13-01-2000	NONE	
WO 9963096 A	09-12-1999	NONE	

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/54, 15/82, A01H 5/00, 5/10, 3/00

A1

(11) International Publication Number: WO 00/36114

(43) International Publication Date: 22 June 2000 (22.06.00)

(21) International Application Number: PCT/CA99/01202

(22) International Filing Date: 16 December 1999 (16.12.99)

(30) Priority Data:
60/112,812 17 December 1998 (17.12.98) US

(71) Applicant (*for all designated States except US*): NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

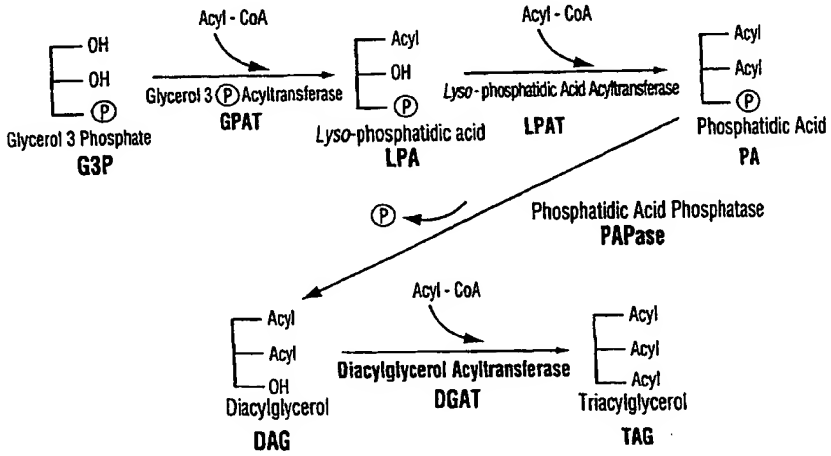
(72) Inventors; and
(75) Inventors/Applicants (*for US only*): ZOU, Jitao [CA/CA]; 1619 Acadia Drive, Saskatoon, Saskatchewan S7H 4T8 (CA). TAYLOR, David, C. [CA/CA]; 622 Wollaston Bay, Saskatoon, Saskatchewan S7J 4C3 (CA). WEI, Yangdou [CA/CA]; 510 Bate Crescent, Saskatoon, Saskatchewan S7H 3A7 (CA). JAKO, Colette, C. [CA/CA]; 1419 7th Avenue N., Saskatoon, Saskatchewan S7K 2W3 (CA).

(74) Agents: GALE, Edwin, J. et al.; Kirby, Eades, Gale, Baker, Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.

(54) Title: DIACYLGLYCEROL ACYLTRANSFERASE GENE FROM PLANTS



(57) Abstract

The present invention relates to the isolation, purification, characterization and use of the plant diacylglycerol acyltransferase (DGAT) gene and genetic products. For example, the invention provides DGAT cDNA [SEQ ID NO:1] (pDGATcDNA; ATCC No PTA–989) and a plant diacylglycerol acyltransferase gene [SEQ ID NO:3] (pDGATgene; ATCC No PTA–988) from the Brassicaceae (specifically Arabidopsis thaliana). The invention includes isolated and purified DGAT DNA, preferably of the stated sequences and homologues, and relates to methods of regulating seed oil content, the ratio of diacylglycerol/triacylglycerol proportions in the seed oil, fatty acid synthesis, seed oil acyl composition, seed size/weight and carbon flux into other seed components, using the gene, and to tissues and plants transformed with the gene. The invention also relates to transgenic plants, plant tissues and plant seeds having a genome containing an introduced DNA sequence of the invention, and a method of producing such plants and plant seeds. The invention also relates to [SEQ ID NO:1] containing an 81 bp insertion [SEQ ID NO:23], and uses thereof to modify oil content, acyl composition of triacylglycerols, seed size or carbon flux into other seed components.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

DIACYLGLYCEROL ACYLTRANSFERASE GENE FROM PLANTS

TECHNICAL FIELD

This invention relates to plant genes useful for the genetic manipulation of plant characteristics. More specifically, the invention relates to the identification, isolation and introduction of diacylglycerol acyltransferase (DGAT) genes useful, for example, for altering the seed oil content, the ratio of diacylglycerol / triacylglycerol proportions in the seed oil, fatty acid synthesis, seed oil acyl composition, seed size/weight and carbon flux into other seed components, in commercial or crop plants.

BACKGROUND ART

Plant seed oils are major sources of essential polyunsaturated fatty acids for human diets and renewable feedstocks for chemical industries. The enzymes of the fatty acid synthase complex in the plastids of developing seeds are responsible for the biosynthesis of fatty acids that are channeled into the cytosolic acyl-CoA pool to sustain triacylglycerol accumulation. Triacylglycerol (TAG) biosynthesis is located in the endoplasmic reticulum with glycerol-3-phosphate and fatty acyl-CoAs as the primary substrates. There are three acyltransferases involved in the plant storage lipid bioassembly, namely the glycerol-3-phosphate acyltransferase (GPAT, EC 2.3.1.15), the *lyso*-phosphatidic acid acyltransferase (LPAT, EC 2.3.1.51) and the diacylglycerol acyltransferase (DGAT, EC 2.3.1.20). These three acyltransferases catalyze the stepwise acylation of the glycerol backbone with the final step being the acylation of *sn*-1, 2-diacylglycerol (DAG) by DGAT into the formation of TAGs, a biochemical process generally known as the Kennedy pathway (Stymne and Stobart, 1987).

Among the three ER-based fatty acyl-CoA acyltransferases, only LPAT gene(s) have been cloned from plants (Knutzon *et al.*, 1995, Lassner *et al.*, 1995). Like several other enzymes involved in storage lipid biosynthesis, acyltransferases are intrinsic ER proteins and are extremely difficult to purify. The research on plant DGAT has been largely limited to studies of activity profiles by using the particulate fractions generated by differential centrifugation of seed or microspore-derived embryo homogenates (Weselake *et al.*, 1993). Although partial purification of

DGAT from cotyledons of germinating soybean seeds was reported (Kwanyuan and Wilson, 1986), detailed molecular characterization of this enzyme is lacking.

Accordingly, while the Kennedy pathway is known and shows the steps in the biosynthesis of TAGs in plants, there has not been any identification and use of a genetic element that can be used reliably in plants to modify TAG synthesis and composition in a way that may be exploited commercially.

DISCLOSURE OF THE INVENTION

An object of the invention is to identify, isolate and clone a genetic element that may be used to modify the natural formation of triacylglycerols in plants in order to increase the yield of commercial plant oils, or to modify their composition to achieve specific commercial improvements of plants and plant products.

Another object of the invention is to identify, isolate and characterize diacylglycerol acyltransferase (DGAT) gene and cDNA sequences from Arabidopsis and to utilize these sequences in the genetic manipulation of plants.

Another object of the invention is to provide a vector containing the full-length DGAT cDNA sequence from *Arabidopsis* in a sense orientation under the control of a seed-specific promoter (e.g. napin; See Josefsson et al., 1987; Radke et al., 1988; Voelker et al., 1992), for re-introducing into *Arabidopsis* or for introducing into other plants.

Another object of the invention is to provide a vector containing a genomic fragment from *Arabidopsis* consisting of the full-length DGAT gene under the control of its own 5' upstream regulatory sequences, for re-introducing into *Arabidopsis* or for introducing into other plants.

Another object of the invention is to provide a method to construct a vector containing the full-length DGAT sequence or a significant portion of the DGAT sequence from *Arabidopsis*, in an antisense orientation under control of either a constitutive or a seed-specific promoter, for re-introducing into *Arabidopsis* or for introducing into other plants.

Another object of the invention is to provide a method of modifiying *Arabidopsis* and other plants to change their seed oil content.

Another object of the invention is to provide a method of modifiying *Arabidopsis* and other plants to change the acyl composition of their seed oil.

Another object of the invention is to provide a method of modifiying *Arabidopsis* and other plants to change their average seed weight or size.

According to one aspect of the present invention, there is provided a vector containing isolated and purified deoxyribonucleic acid (cDNA) of SEQ ID NO:1 (pDGATcDNA; ATCC No PTA-989), for introduction of the cDNA in a sense orientation into a plant cell.

As another aspect of the present invention, there is provided a vector containing isolated and purified genomic deoxyribonucleic acid (genomic DNA) of SEQ ID NO:3 (pDGATgene; ATCC No PTA-988), for introduction of the gene in a sense orientation into a plant cell.

According to yet another object of the invention, there is provided a method for preparing a vector containing SEQ ID NO:1 or a part thereof, for introduction of the gene or partial gene in an antisense orientation, into a plant cell.

According to yet another object of the invention, there is provided seed of *Arabidopsis thaliana* ecotype Columbia mutant AS11 (ATCC No. PTA-1013) and characterization of its lipid phenotype (Katavic et al., 1995; Zou et al. 1999). The AS11 mutant seed line has an insertion mutation at the *TAG1* locus on chromosome II, and produces plants exhibiting reduced DGAT activity (Figure 4) and an reduced TAG/DAG ratio during seed development (Table 1), resulting in an altered seed fatty acyl composition (Figure 2), reduced oil content (Table 1), and increased seed oil diacylglycerol content during development (Figure 3) and at maturity (lower TAG/DAG ratio *cf* Table 1). The cDNA sequence of the AS11 DGAT is shown in SEQ ID NO:23, the genomic DNA sequence is shown in SEQ ID NO:24 and the translated protein sequence of the AS11 DGAT is shown in SEQ ID NO:25.

The invention also relates to transgenic plants and plant seeds having a genome containing an introduced DNA sequence of SEQ ID NO:1 or SEQ ID NO:3, and a method of producing such plants and plant seeds.

The invention also relates to SEQ ID NO:1 or SEQ ID NO:3, or a part of SEQ ID NO:1 or SEQ ID NO:3, or SEQ ID NO:1 containing an 81 bp insertion [SEQ ID NO: 23] or SEQ ID NO:3 containing an 147 bp insertion [SEQ ID NO:24] such that the deduced amino acid sequence of the encoded protein contains the repeated sequence SHAGLFNLCVVVLIAVNSRLIIENLMK [SEQ ID NO:25] where the spacing and identity of the underlined amino acids are identical or are replaced by conserved substitutions, characterized in that said sequence has been introduced in sense or antisense orientation, and a method of producing such plants and plant seeds.

As will be appreciated by persons skilled in the art, the invention also relates to substantially homologous DNA sequences from plants encoding proteins with deduced amino acid sequences of 25% or greater identity, and 40% or greater similarity, isolated and/or characterized and/or designed by known methods using the sequence information of SEQ ID NO:1 or SEQ ID NO:3 or or SEQ ID NO:1 containing an 81 bp insertion [SEQ ID NO:23] such that the deduced amino acid sequence of the encoded protein contains the repeated sequence SHAGLFNLCVVVLIAVNSRLIIENLMK (SEQ ID NO:25) where the spacing and identity of the underlined amino acids are identical or are replaced by conserved substitutions, and to parts of reduced length that are still able to function as inhibitors of gene expression by use in an anti-sense, co-suppression (Transwitch; Jorgensen and Napoli 1994) or other gene silencing technologies. It will be appreciated by persons skilled in the art that small changes in the identities of nucleotides in a specific gene sequence may result in reduced or enhanced effectiveness of the genes and that, in some applications (e.g. anti-sense or co-suppression), partial sequences often work as effectively as full length versions. The ways in which the gene sequence can be varied or shortened are well known to persons skilled in the art, as are ways of testing the effectiveness of the altered genes. All such variations of the genes are therefore claimed as part of the present invention.

Other preferred degrees of identity to the indicated sequences are at least 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95%; and other preferred degrees of similarity are at least 50%, 60%, 70%, 80%, 90% and 95%. The inventors have used a computer program known as MegAlign®, DNASTAR® of DNASTAR Inc., 1228 South Park Street, Madison, WI 53715, USA, for assessing homology. This program is based on the Clustal V algorithm (Higgins and Sharp (1998): A package for performing multiple sequence alignment on a microcomputer; GENE 73:237-244). For each gap introduced in the alignment, the program deducts a penalty from the score. A higher gap penalty suppresses gapping; a lower value promotes it. The program also assesses penalties based on the length of the gap. The more residues the gap spans, the greater the penalty. The program deducts these penalties from the overall score of the alignment.

Stated more generally, the present invention relates to the isolation, purification and characterization of a diacylglycerol acyltransferase (DGAT) gene from the *Brassicaceae* (specifically *Arabidopsis thaliana*) and demonstrates its utility in regulating fatty acid synthesis, seed oil content, diacylglycerol/triacylglycerol ratios and seed size/weight. Until now, no concrete data is available on the gene structure of plant DGATs, or their utility in altering oil content or composition through genetic manipulation.

When considering altered oil contents or compositions, results from averages of statistically-significant numbers of plants or seeds according to the invention are best compared with results from averages of statistically-significant numbers of untransformed (control) plants or seeds of the same genotype grown under identical conditions at the same time. This allows for the variability of individual plants of the same genotype, particularly when such plants are grown under different conditions. The actual number of plants or seeds used to form the required average may vary, but should be enough to provide a generally constant average whenever such number is selected. Generally, the number should be at least 10, and is more preferably at least 20, 30, 50 or 100.

The DGAT gene was cloned, characterized and authenticated from *Arabidopsis* by: (1) selection and characterization of plant ESTs sharing some homology to mammalian acyl-CoA: cholesterol acyltransferases; (2) the functional

expression of a full-length cDNA in yeast; (3) the characterization and isolation of the DGAT (*TAG1*) gene from Arabidopsis mutant AS11 containing an insertion mutation in the DGAT gene and a seed oil phenotype which consists of an altered DAG/TAG ratio, and an altered oil content and acyl composition; (4) complementation of the AS11 mutant by insertion of the DGAT cDNA sequence to restore a wild-type fatty acid composition; (5) the over-expression of the DGAT cDNA in wild-type *A. thaliana* transgenic plants which produce seeds with an increased oil content, increased average seed weight and altered seed oil acyl composition.

The *A. thaliana* DGAT structure is significantly homologous (over 40% identical over a region of more than 400 amino acids) to its mammalian counterparts, and is highly homologous to subsequently reported putative *B. napus* DGATs at both the nucleotide (92%) and the deduced amino acid (90%) levels (Nikyiforuk et al, 1999; GenBank/EMBL Accession No AF155224; AF164434).

The DGAT of the current invention is useful in manipulating DGAT activity, and triacylglycerol bioassembly in plants. For example, by transforming plants with a construct containing the DGAT gene in a sense orientation, under the control of a tissue-specific promoter (e.g. seed-specific promoter napin), the expression of DGAT and accumulation of seed oil can be enhanced or the acyl composition of the seed oil altered. Yet another example would be to express the DGAT cDNA under the control of a constitutive promoter (e.g. 35S (Datla et al., 1993)) to increase the TAG content of vegetative tissues (leaves, roots, stems). This may have particular advantages for altering the starch/oil ratio in root crops.

Alternatively, DGAT expression can be silenced to some degree by anti-sense or co-suppression (Transwitch) phenomena (De Lange et al., 1995; Mol et al., 1990; Jorgensen and Napoli, 1994; Kinney, 1995; Vaucheret et al, 1998; Taylor, 1998). For example, silencing DGAT in a seed specific manner may result in a reduction in TAG accumulation. This could have applications in reducing the oil content in seed barley to enhance stability during storage. As a second example, seed-specific silencing may lead to a relatively high accumulation of DAG or an increase in the DAG/TAG ratio in the developing or mature seed. As yet another example, the expression of the mutated DGAT gene which results in a 27 amino

acid repeat insertion in the mutant DGAT protein (See Figure 5 a) can be used to alter the DAG/TAG ratio in developing and mature seed. Such manipulations can lead to edible seed oils produced naturally in the plant, containing enhanced relative levels of DAG /reduced levels of TAG (See Figure 3; Table 1) to act as all-natural emulsifiers in the food and confections industries, or to enhance the nutritional/health profile of vegetable oils as functional foods (e.g. as cooking oils, stir fry oils, in salad dressings, margarines etc.) by inhibiting neutral fat deposition in humans. Processed oils produced from canola and soybean which contain increased proportions of diacylglycerol have been cited by the Kao Corporation of Japan (e.g. Econa Cooking Oil; Kao Corporation KI, 1-14-10 Nihonbashi-Kayabacho, Chuoku, Tokyo 103 Japan; e-mail: 210064@kastanet.kao.co.jp) as a product making it difficult for blood neutral fat to increase after a meal, and for fat to cling to the body, thereby assisting individuals who are overweight or who suffer from high neutral fat levels. As a third example, silencing or reducing the activity of DGAT in a seed specific manner (as observed in mutant AS11; e.g. by over-expressing SEQ ID NO:23 or silencing expression of SEQ ID NO:1 or SEQ ID NO:3), and combining this trait with the capacity to produce polyhydroxyalkanoates (PHAs; e.g. polyhydroxybutyrate) in seeds (Poirier et al., 1992; 1995) will allow an increased flow of unesterified fatty acids towards β-oxidation (Poirier et al., 1999). By recycling or diverting the unesterified fatty acids into β oxidation, the resulting acetyl-CoA moieties will lead to a significant increase in polyhydroxyalkanoates (PHAs) or a change in PHA composition (Poirier et al., 1999). Transgenic plants producing PHAs in seeds have potential for utility as biodegradeable thermoplastics. However, up to now, the levels of PHAs produced have been relatively small (Poirier et al, 1992; 1995). The utility of transgenically reducing the DGAT activity to significantly enhance PHA production (e.g. 10-fold increase) in PHA-producing seeds is now possible, due to the current DGAT invention.

Some of the manipulations and deliverables which are possible using the DGAT gene or a part therof, include, but are not limited to, the following: seeds with increased or decreased oil content; seeds containing oils with an enhanced diacylglycerol content, seed oils with an altered acyl composition; plants producing larger or heavier seeds; plants exhibiting an enhanced or altered capacity to accumulate storage compounds in other storage organs (e.g. tubers, roots).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram illustrating the Kennedy pathway for the bioassembly of triacylglycerols in plants, and shows the critical role played by DGAT as the final step of the Kennedy pathway.

Figure 2 is a graph showing the comparison of the fatty acid composition of seed oil from wild-type (WT) and DGAT mutant AS11 lines of *Arabidopsis thaliana*. Proportions of fatty acids are reported as Mol % of the total fatty acid composition of the seed oil from each line. Error bars are ± SE (n= 10 plants of each line sampled, with 50 seeds per sample per analysis).

Figure 3 is a graph showing a comparison of the DAG content in developing seed of wild-type (WT) and DGAT mutant AS11 lines of *Arabidopsis thaliana*. More specifically, it is a comparison of the fatty acid content of DAG pool in wild-type green developing seeds compared to that of the AS11 mutant.

Figure 4 is a graph showing a comparison of the DGAT activity (pmol/min/mg protein) in developing seeds at the milky, early green, mid-green and brown-green stages of embryo development in wild-type (WT) and DGAT mutant AS11 lines of *Arabidopsis thaliana*. Developing seeds at each stage were selected and DGAT enzyme analyses conducted as described previously by Katavic et al., 1995).

Figure 5(a) shows the amino acid sequence alignment of the *Arabidopsis* DGAT (AtTAG1) [SEQ ID NO:2] with mammalian (mouse and putative human) DGATs. MDGAT, mouse DGAT [SEQ ID NO:4]; GenBank/EMBL Accession No. AF078752 (Cases *et al.*, 1998)]; HARGP1, human ARGP1 protein [SEQ ID NO:5]; GenBank/EMBL Accession No. AF059202; Oelkers et al., 1998]. Dots indicate gaps. Identical amino acid residues are highlighted in black. Conserved residues are shaded. The 27-amino acid repeat found in *Arabidopsis thaliana* mutant AS11 and generated by the insertion mutation (81 bp) found in SEQ ID NO:23 (SHAGLFNLCVVVLIAVNSRLIIENLMK) is overlined thus: ----------. The putative diacylglycerol binding site is overlined thus: ==========. The SnRK1 targeting site is overlined thus: _____ with an asterisk (*) over the serine (S) residue as the targeted phosphorylation site.

Figure 5(b) shows the Kyte-Doolittle hydropathy plot of the DGAT protein.

Figure 6(a) shows the results of a Northern analysis of *TAG1* gene expression in *Arabidopsis thaliana*. Total RNA was extracted from roots (RT), leaves (LF), flowers (FL), young seedlings (YS), developing siliques (SL), and germinating seeds (GS).

Figure 6(b) shows the results of Southern blot analysis of the *TAG1* gene in *Arabidopsis thaliana*. Genomic DNA was digested with restriction enzymes BglII (Lane 1), EcoRI (Lane 2), and HindIII (Lane 3). The *TAG1* DNA probe was ^{32}P labeled by random priming.

Figure 7(a) is a diagrammatic representation of the *TAG1* gene structure. The boxes indicate the 16 exons (solid boxes for coding regions, open box for untranslated regions), and the lines represent the 15 introns. A, B and C denote the positions of the primers used for PCR amplifications of the segments from wild type (WT) and AS11. The specific primers A, B and C are described in Experimental Procedures: *Primer Strategy* (found later in this specification).

Fig 7(b) shows gel separation of the PCR products amplified from wild type (WT) and AS11. Lane 1, PCR product with primers A and B using WT genomic DNA as template. Lane 2, PCR product with primers A and B using AS11genomic DNA as template. Lane 3, PCR product with primers C and B using WT genomic DNA as template. Lane 4, PCR product with primers C and B using AS11 genomic DNA as template. Lane 5, RT-PCR with primers A and B using RNA prepared from WT seedling RNA. Lane 6, RT-PCR with primers A and B using RNA prepared from AS11 seedling RNA.

Figure 8 is a graph showing microsomal DGAT activity in Yeast Strain YMN5 Transfomred with empty plasmid (pYES Con) and with the *A. thaliana* DGAT cDNA (pYES:DGAT). This illustrates the expression of the *TAG1* cDNA in yeast. Host cultures of strain YMN5 were transformed with *pYES2* plasmid only (*pYES2*; without *TAG1* insert) or with *pYES2* containing the *TAG1* cDNA insert (*pYES2:TAG1*). Following induction in the presence of galactose, transformants were lysed and assayed for DGAT activity as described in the Experimental Procedures. The results of two separate DGAT activity experiments are shown.

Figure 9 is a map of plasmid pSE129A which may be used as a vector. The vector contains the following salient features for plant transformation in the current invention: the seed-specific napin promoter and NOS terminator between which is a multiple cloning site.

Figure 10 is a graph showing the complementation of the AS11 DGAT mutation with the wild-type cDNA. Transformation of *Arabidopsis thaliana* mutant line AS11 with the DGAT cDNA [SEQ ID NO:1] under the control of a napin promoter, leads to a restoration of the wild-type (WT) fatty acid composition in the seed oil of the transformant lines 3-4, 9-1, 14-2 and 9-4. Fatty acid composition (wt %) was determined on the seed oil extracted from 100-seed samples from *A. thaliana* non-transformed controls (WT), mutant line AS11, and T_2 seeds of napin:DGAT transgenic lines.

Figure 11 is a graph showing the seed oil content of non-transformed WT control, and pRD400 control (empty plasmid) and napin:DGAT T_2 transgenic *Arabidopsis thaliana* seed lines. More particularly, the graph shows the transformation of wild type (WT) *Arabidopsis thaliana* with the DGAT cDNA [SEQ ID NO:1] under the control of a napin promoter, leads to a higher seed oil content in the DGAT transgenic lines. Oil content is expressed as μg total fatty acids (FAs) per 100 seeds from *A. thaliana* non-transformed controls (WT Con), and T_2 seeds of pRD400 control (empty plasmid) transgenic, and napin:DGAT transgenic lines 1', 2', 9, 10 and 11. Standard error bars for the control lines are indicated; n=10.

Figure 12 is a graph showing the average 100-seed weight of non-transformed WT control, and pRD400 control (empty plasmid) and napin:DGAT T_2 transgenic *Arabidopsis thaliana* seed lines. More specifically, the graph shows the over-expression of the DGAT cDNA under the control of a napin promoter, in wild-type (WT) *Arabidopsis thaliana* leads to a higher average seed weight. The average weight of 100-seed samples from *A. thaliana* non-transformed controls (WT Con), and T_2 seeds of pRD400 control (empty plasmid) transgenic, and napin:DGAT transgenic lines 1', 2', 9, 10 and 11 are reported as mg dry weight (DW).

Figure 13 is a graph showing the positive correlation between oil content (expressed as μg Total fatty acids (FAs) per 100 seeds) and average seed weight (expressed as average mg DW per 100-seed samples) from A. thaliana non-

transformed controls (WT Con ◆), and T2 seeds of pRD400 control (empty plasmid) transgenic (() and napin:DGAT transgenic lines 1', 2', 9, 10 and 11 (■) are reported as mg dry weight (DW).

BEST MODES FOR CARRYING OUT THE INVENTION

Fig. 1 is a diagram illustrating the Kennedy pathway for the biosynthesis of TAGs in plants. Of the various illustrated enzymes, DGAT is the only enzyme in the Kennedy pathway that is exclusively committed to TAG biosynthesis, and its key role is apparent from the scheme of Figure 1. *sn*-1,2-DAG, generated as a result of either the catalytic action of PA phosphatase (EC 3.1.3.4) or CPTase (EC 2.7.8.2), can be used in the biosynthesis of TAG.

For this reason, the inventors of the present invention decided to investigate DGAT to see if the corresponding gene in plants could be sequenced and cloned and used to modify the seed oil content and fatty acid composition of plants in a way that could be commercially useful.

The acyl-CoA dependent acylation of *sn*-1,2-DAG is catalyzed by DGAT (Stymne and Stobart, 1987). In developing and germinating seeds of oilseed plants, TAG accumulation and DGAT activity have been shown to associate with the endoplasmic reticulum (ER; high speed microsomal fraction) (Stobart et al., 1986; Cao and Huang, 1986; Stymne and Stobart, 1987; Frentzen, 1993; Settlage et al, 1995; Lacey and Hills, 1996). The biochemical properties of microsomal DGAT have been examined in a number of plant systems (Frentzen, 1993) including developing seeds (Bernerth and Frentzen, 1990; Vogel and Browse, 1996; Cao and Huang, 1987) and embryo cultures (Taylor et al., 1991; Weselake et al., 1991; Taylor et al., 1992; Little et al., 1994) of *B. napus* L. In general, studies with developing seeds indicate that DGAT activity increased rapidly during the active phase of oil accumulation and then decreases markedly as seed lipid content reaches a plateau (Tzen et al., 1993; Weselake et al., 1993).

A number of studies with both mammalian (Mayorek et al., 1989; Tijburg et al., 1989) and plant (Ichihara et al., 1988; Perry and Harwood, 1993 a and 1993 b; Settlage et al. 1995) systems have suggested that DGAT may catalyze a rate-limiting reaction in TAG bioassembly. However, this hypothesis has not been

rigorously tested, and has not been reduced to practice by transgenic expression of any DGAT gene in plant or animal systems, until now. Developing seeds of *B. napus* L., cv Shiralee, have been shown to produce significant levels of DAG during the active phase of oil accumulation suggesting that DGAT catalyzed reaction may regulate the flow of carbon into TAG (Perry and Harwood, 1993 a and 1993 b). In addition, an ethyl methanesulfonate-induced (EMS-induced) mutant of *A. thaliana*, designated AS11, has been shown to have a reduced DGAT activity that correlated with both an increased DAG pool and decreased accumulation of TAG (Katavic et al. 1995). Given its possible rate-limiting role in TAG bioassembly, the inventors of the present invention have identified DGAT as a potential target in the genetic modification of plant lipid biosynthesis.

Previously, the partial characterization of an EMS-induced *Arabidopis thaliana* mutant, AS11, with altered fatty acid composition was reported (Katavic *et al.*, 1995). In comparison to wild type plant seeds, AS11 seeds have reduced levels of the very long chain fatty acid eicosenoic acid (20:1) and reduced oleic acid (18:1) and accumulate α-linolenic acid (18:3) as the major fatty acid in triacylglycerols (Figure 2). The AS11 mutant has a consistently lower ratio of TAG/DAG in developing seeds, and it accumulates an elevated amount of seed DAG (Figure 3), the substrate of the diacylglycerol acyltransferase. Through a series of biochemical analyses, it was shown that AS11 had reduced diacylglycerol acyltransferase activities throughout seed development (Figure 4). AS11 also had a reduced (by 25-30%) oil content phenotype, providing some evidence that DGAT may be controlling flux into TAG biosynthesis, as shown in Table 1 below. The AS11 did not have a wrinkled-seed phenotype as described in other low-seed-oil mutants (Focks and Benning, 1998).

Table 1

Comparison of AS11 [Katavic et al., (1995) and wild-type A. thaliana seeds with respect to lipid profiles at mid-development, and the relative TAG, DAG and sterol ester contents in AS11 and WT seeds at maturity.

Seed Type	TAG/DAG ratio at mid-development[a]	TAG/DAG ratio at maturity[a]	Relative TAG content at maturity[b] [c](nmol/mg DW)	Sterol Esters at maturity (% of Totol Lipid Extract)[d]
WT	17	90	1.00[b] [c](255)	0.8
AS11	5	20	0.6[b] [c](174)	1.15

[a] Embryos staged and lipids measured as described in Katavic et al., (1995); [b] Relative TAG content of 200-seed samples of AS11 and WT were meaured by MASS-1H-NMR according to the method of Rutar (1989). The integration response for resonances attributabe to liquid-like oil were summed and the value for AS11 seed is reported relative to the response for the WT control seed sample (the latter set at a value of 1.00); [c] TAG content (nmoles/mg DW) measured by transmethylation of a TLC-purified TAG fraction, followed by GC analysis of fatty acid methyl esters; [d] A total lipid extract was prepared as dedscribed by Taylor et al., (1991; 1992), and sterol esters isolated and characterized as described in the Experimental Procedures.

Genetic analysis indicated that the fatty acid phenotype is caused by a semidominant mutation in a nuclear gene, designated *TAG1*. The mutation was mapped to chromosome II, and was estimated to lie in the region approximately 17.5 $\pm$ 3 cM from the *sti* locus and 8 $\pm$ 2 cM from the *cp2* locus.

Because a DGAT gene has not heretofore been cloned from any plant, until now, it has not been possible to address the possibility of genetic modifications to alter carbon flux, increase fatty acid synthesis, oil content, oil acyl composition, or seed size, by modulating plant DGAT activity.

However, there are many examples of successful modifications to plant metabolism that have been achieved by genetic engineering to transfer new genes or to alter the expression of existing genes, in plants. It is now routinely possible to introduce genes into many plant species of agronomic significance to improve crop performance (e.g. seed oil or tuber starch content/composition; meal improvement;

herbicide, disease or insect resistance; heavy metal tolerance etc.) (MacKenzie and Jain, 1997; Budziszewski et al., 1996; Somerville, 1993; Kishore and Somerville, 1993).

For example, increases in the proportions of some strategic fatty acids and in the quantities of seed oil have been achieved by the introduction of various fatty acid biosynthesis and acyltransferase genes in oilseed crops. These include the following demonstrations: Expression of an anti-sense construct to the stearoyl-ACP Δ9 desaturase in Brassicaceae led to an increase in the stearic acid content (Knutzon et al., 1992). Expression of a medium chain fatty acyl-ACP thioesterase from California Bay, in Brassicaceae was demonstrated to increase the lauric acid (12:0) content (Voelker et al., 1992; 1996). Expression of a Jojoba β keto-acyl-CoA synthase in low erucic acid Brassicaceae led to an increase the level of erucic acid (22:1); the effect following expression in high erucic acid cultivars was negligible (Lassner et al., 1996). Increased proportions of oleic acid in Brassica napus and in soybean have been achieved by silencing the microsomal FAD2 (Δ12) desaturase (Hitz et al., 1995; Kinney, 1995 ; 1997). Transformation of Arabidopsis thaliana and rapeseed (B. napus) with a yeast sn-2 acyltransferase resulted in seed oils with increased proportions of 22:1 and other very long-chain fatty acids and significant increases in seed oil content (Zou et al., 1997).

Starch deposition has also been altered by genetic engineering. By expression of a mutant E. coli glgC16 gene encoding an ADP glucose pyrophosphorylase in potato tubers, an increase in starch accumulation was achieved (Stark et al., 1992).

The inventors therefore considered the DGAT gene to hold great promise for the desired modification of TAGs in plants.

The best modes for carrying out the invention will be apparent from the following description of the results of tests and experiments that have been carried out by the inventors.

The inventors chose to use the well-accepted model plant system *Arabidopsis thaliana* for the cloning of DGAT, as a host system for genetic engineering to alter DGAT expression, and to study the effects of altering DGAT

expression on seed triacylglycerol bioassembly. This is because, over the past several years, *Arabidopsis thaliana*, a typical flowering plant, has gained increasing popularity as a model system for the study of plant biology. As a result of the ease with which this plant lends itself to work in both classical and molecular genetics, *Arabidopsis* has come to be widely used as a model organism in plant molecular genetics, development, physiology and biochemistry (Meyerowitz and Chang, 1985; Meyerowitz, 1987; Goodman et al., 1995). This model dicotyledonous plant is also closely related to *Brassica* crop species and it is increasingly apparent that information concerning the genetic control of basic biological processes in *Arabidopsis* will be transferable to other species (Lagercrantz et al., 1996).

Indeed, there are numerous examples wherein studies of the molecular biology and biochemistry of a particular metabolic pathway or developmental process and the possibility of genetically engineering a plant to bring about changes to said metabolic pathway or process, has first been tested in the model plant *Arabidopsis*, and then shown to yield similar phenotypes in other plants, particularly crop plants.

For example, the extra- plastidial membrane associated oleate (18:1) Δ12 (ω-6) desaturase gene, *FAD2*, was originally studied and eventually cloned from *Arabidopsis thaliana,* by identifying the lesion found in an *A. thaliana* mutant defective in desaturating oleate to produce linoleate (18:2) on the phosphatidylcholine backbone. This resulted in a high oleic acid phenotype in the *A. thaliana* seed oil (Okuley et al., 1994). Genetic engineering of both soybean (*Glycine max.*) and canola *B. napus* to silence the indigenous *FAD2* gene(s) in a seed-specific manner by anti-sense or co-suppression approaches, resulted in similar high oleic acid seed oil phenotypes (Kinney, 1995; 1997).

Transgenic expression of a yeast *sn*-2 acyltransferase (*SLC1-1*) gene to achieve enhanced seed oil and very long-chain fatty acid content was first performed in *Arabidopsis* and later shown to yield similar phenotypes in transgenic rapeseed (*B. napus*) experiments (Zou et al., 1997). *Arabidopsis thaliana* has repeatedly shown itself to be a useful model system for metabolic engineering of metabolic pathways (e.g. lipid biosynthesis, photosynthesis) or processes (organogenesis, reproductive development etc.) common to all higher plants.

In the area of secondary metabolism/signal transduction, an anthocyanin pathway-specific transcriptional activator from the monocot maize designated as *R* (the *myc* transcription factor involved in activation of biosynthetic genes for anthocyanin production in the aleurone cells of maize kernels), was expressed in the dicot *Arabidopsis*, causing augmented anthocyanin pigmentation in the infloresecences. Subsequent expression in another dicot, tobacco (*Nicotiana tabacum*), resulted in similar floral pigmentation changes (Lloyd et al., 1992). These experiments demonstrate that whole pathways common to all flowering plants can be co-ordinately controlled through the introduction of transcriptional regulators, and that the mechanisms are common to diverse plant species.

In the context of the current invention, all plant seeds accumulate some triacylglycerol (oil) and this ubiquitous process is affected, at least in part, by the activity of a microsomal DGAT, as explained previously. Thus, many of the effects observed following genetic engineering to modulate DGAT expression in *Arabidopsis* can be expected and predicted to result in similar phenotypes when carried out in all other plants. For example, after the present invention was made, information has become available that supports the findings of the present inventors by showing that *B. napus* has a highly homologous DGAT gene (Nikiforuk et al., 1999), and thus *B. napus* is a clear target for similar genetic modifications as those shown for *A. thaliana*.

There are a number of ways by which genes and gene constructs can be introduced into plants, and a combination of plant transformation and tissue culture techniques have been successfully integrated into effective strategies for creating transgenic crop plants. These methods, which can be used in the present invention, have been extensively reviewed elsewhere (Potrykus, 1991; Vasil, 1994; Walden and Wingender, 1995; Songstad et al., 1995), and are well known to persons skilled in the art. For example, one skilled in the art will certainly be aware that, in addition to *Agrobacterium*-mediated transformation of *Arabidopsis* by vacuum infiltration (Bechtold et al., 1993) or wound inoculation (Katavic et al., 1994), it is equally possible to transform other plant and crop species, using *Agrobacterium* Ti-plasmid-mediated transformation (e.g. hypocotyl (DeBlock et al., 1989) or cotyledonary petiole (Moloney et al, 1989) wound infection), particle bombardment/biolistic methods (Sanford et al., 1987; Nehra et al., 1994; Becker et

al., 1994) or polyethylene glycol-assisted protoplast transformation (Rhodes et al., 1988; Shimamoto et al., 1989) methods.

As will also be apparent to persons skilled in the art, and as extensively reviewed elsewhere (Meyer, 1995; Datla et al., 1997), it is possible to utilize plant promoters to direct any intended up- or down-regulation of transgene expression using constitutive promoters (e.g. those based on CaMV35S), or by using promoters which can target gene expression to particular cells, tissues (e.g. napin promoter for expression of transgenes in developing seed cotyledons), organs (e.g. roots), to a particular developmental stage, or in response to a particular external stimulus (e.g. heat shock).

Particularly preferred plants for modification according to the present invention include *Arabidopsis thaliana*, borage (*Borago* spp.), Canola, castor (*Ricinus communis*), cocoa bean (*Theobroma cacao*), corn (*Zea mays*), cotton (*Gossypium* spp), *Crambe* spp., *Cuphea* spp., flax (*Linum* spp.), *Lesquerella* and *Limnanthes* spp., Linola, nasturtium (*Tropaeolum* spp.), *Oenothera* spp., olive (*Olea* spp.), palm (*Elaeis* spp.), peanut (*Arachis* spp.), rapeseed, safflower (*Carthamus* spp.), soybean (*Glycine* and *Soja* spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.), *Vernonia* spp., wheat (*Triticum* spp.), barley (*Hordeum* spp.), rice (*Oryza* spp.), oat (*Avena* spp.) sorghum (*Sorghum* spp.), rye (*Secale* spp.) or other members of the *Gramineae*.

The present invention is particularly useful when used to modify the yield or composition of oilseed produced from oilseed crops. Oilseed crops are plant species that are capable of generating edible or industrially useful oils in commercially significant yields, and include many of the plant species listed above. Such oilseed crops are well known to persons skilled in the art.

RESULTS

Isolation of the TAG1 (DGAT) cDNA from Arabidopsis thaliana

Since one of the most likely defects in AS11 mutant is at the DGAT itself (Table 1; Fig 4), the inventors attempted cloning strategies based on sequence information of enzymes that share common substrates with DGAT. One of the

candidate enzymes that would serve this purpose is the acyl-CoA: cholesterol acyltransferase (ACAT, EC 2.3.1.26) (Chang *et al.*, 1997). Like DGAT, ACAT is an ER protein functioning as an *O*-acyltransferase by using acyl-CoA as the fatty acyl donor for the esterification of free cholesterol to generate sterol esters. Through a BLAST database search, the inventors identified an *Arabidopsis thaliana* expressed sequence tag (EST) [accession no. AA042298; SEQ ID NO:6] with a deduced amino acid sequence showing 41 % identity to that of the yeast acyl-CoA: cholesterol acyltransferase (Yang *et al.*, 1996, Yu *et al.*, 1996), within the short sequence (104 amino acids) that was available for the EST.

The corresponding cDNA (E6B2T7) clone was obtained from the Arabidopsis Biological Resource Center, Columbus, Ohio. Upon complete sequencing, the 878 bp E6B2T7 clone was found to be a partial cDNA. However, the ORF prediction from this partial cDNA confirmed the initial EST search results in that the encoded product is structurally similar to ACAT, especially in the regions at the *C*-terminus. The inventors were confident that the cDNA contained the 3' untranslated region through an ORF search, although the polyA tail was missing.

The inventors further used the partial cDNA sequence to search against *Arabidopsis thaliana* genomic sequence information. An *Arabidopsis* 'IGF' BAC clone 'F27F23' [accession no. AC003058] was identified to include a region that matched the cDNA, and therefore it was concluded that this was the region encompassing the corresponding gene. Moreover, this BAC clone 'F27F23', is contained in the YAC clone, CIC06E08, which, according to the published map position (http://weeds.mgh.harvard.edu/goodman/c2_b.html), represents a region between centimorgan 35.9 and centimorgan 38.7 on chromosome II; this position is similar to the estimated location for *TAG1*, and the lesion identified by the mutation in AS11 (Katavic *et al.*, 1995). In view of our previous results on the characterization of the AS11 mutant, the map position of this gene strongly suggested that it may encode a DGAT.

To clone a full-length cDNA, a series of oligonucleotide primers were designed, based on the genomic sequences located at different positions 5' upstream of the region covering the partial cDNA. We used these primers in combination with a primer located at the 3' UTR of the partial cDNA (E6B2T7) to

perform PCR reactions with cDNA phagemid prepared from an Arabidopsis thaliana (ecotype Columbia) silique-specific cDNA library (Giraudat et al., 1992) as a template. The longest cDNA amplified was 1904 bp, which we subsequently designated as TAG1, and deposited into the Genbank under accession AJ238008 [SEQ ID NO:1]. We believe this cDNA represents a full-length clone because its size is in agreement with that of the transcript detected in the northern blot (see Figure 6 a). The longest open reading frame is flanked by a 134-nucleotide 5' untranslated region and a 203-nucleotide 3' untranslated region. There is an in-frame stop codon (TGA at position nt-43) which is followed by an in-frame ATG at position nt-139. It is thus inferred that the ATG at position nt-139 is the initiation codon.

The primary structure of TAG1 predicts a DGAT-related enzyme

The predicted open reading frame of the *TAG1* cDNA encodes for a polypeptide of 520 amino acids with a calculated molecular weight of 58993 Daltons. With the BLAST search program (Altschul *et al.* 1990), it was found that the recently reported mouse diacylglycerol acyltransferase [accession no. AF078752] (Cases *et al.*, 1998) is a protein which showed the highest sequence similarity to the deduced amino acid sequence of *TAG1* (Figure 5a). TAG1 was also similar to a human acyl CoA: cholesterol acyltransferase-related enzyme [accession no. AF059202]. The human acyl CoA: cholesterol acyltransferase-related enzyme, also known as ARGP1, is most likely to be a DGAT with no significant ACAT activity, although the true nature of the enzyme awaits further confirmation (Oelkers *et al.*, 1998). The similarity between TAG1 and the mammalian DGAT extends over a region of more than 400 amino acids with a sequence identity of about 41%. A putative diacylglycerol/phorbol ester-binding motif, HKW-X-X-RH-X-Y-X-P, a signature sequence observed to be unique to DGAT while absent in the ACATs (Oelkers *et al.*, 1998), is located at amino acids 414-424 ([SEQ ID NO: 7]; Figure 5 a). This diacylglycerol binding motif is also found in the subsequently published *B. napus* DGAT sequences (Nikyiforuk et al, 1999; GenBank /EMBL Accession Nos. AF155224, SEQ ID NO:8; AF164434, SEQ ID NO:9). Among other cloned acyltransferases (e.g. GPATs, LPATs, dihydroxyacetone phosphate acyltransferases) it has been reported that there is an invariant proline in a highly hydrophobic block IV that may participate in acyl-CoA

binding (Lewin et al., 1999). In the TAG1 sequence, the hydrophobic block from residues 221-229 containing an invariant proline at residue 224, might constitute such a motif.

TAG1 showed some sequence similarity to other acyl CoA: cholesterol acyltransferases from a number of species (Chang *et al.*, 1997). However, the similarity is largely confined to the *C*-terminus and is lower (around 30%) than is the similarity of TAG1 to the mammalian DGAT.

The TAG1 protein has multiple hydrophobic domains (Figure 5b) and an analysis by the PC Gene program predicted that the protein has 5 possible transmembrane segments (amino acids 178-195, 233-253, 363-388, 433-476, 486-507). In the mammalian DGAT, a putative tyrosine phosphorylation motif was observed (Cases *et al.*, 1998), but no apparent tyrosine phosphorylation site could be found in TAG1. However, a visual examination revealed a consensus sequence ($X\text{-}L^{200}\text{-}X\text{-}K^{202}\text{-}X\text{-}X\text{-}S^{205}\text{-}X\text{-}X\text{-}X\text{-}V^{209}$; SEQ ID NO:10) identified as a targeting motif typical of members of the SnRK1 protein kinase family, with serine residue 205 being the residue for phosphorylation. The SnRK1 (SNF1-related protein kinase-1) proteins are a class of Ser/Thr protein kinases that have been increasingly implicated in the global regulation of carbon metabolism in plants (Halford and Hardie, 1998). This concensus SnRK1 targeting motif is also found in the subsequently published *B. napus* DGAT sequences (Nikyiforuk et al, 1999; GenBank /EMBL Accession Nos. AF155224; AF164434). Interestingly, similar SnRK1 targeting motifs could also be identified in the *lyso*-phosphatidic acid acyltransferases (LPATs) from coconut (Knutzon *et al.*, 1995) and meadowfoam (Lassner *et al.*, 1995), respectively.

The TAG1 gene is ubiquitously expressed in Arabidopsis

Northern blot analyses were performed to investigate the expression profile of the *TAG1* gene. Total RNA was extracted from different tissues, including roots, leaves, flowers, developing siliques, young seedlings and germinating seeds. The highest steady-state level accumulation of *TAG1* transcript was in RNA isolated from germinating seeds and young seedlings (Figure 6a). *TAG1* transcripts were also detected in root, leaf and flower tissues, albeit at lower levels. Surprisingly, the *TAG1* gene is expressed in developing siliques at a level that is comparable to that

of other vegetative tissues, but lower than that of germinating seeds and young seedlings. This expression profile in general is not inconsistent with the notion that DGAT is present in all plant tissues capable of TAG biosynthesis (Kwanyuan and Wilson, 1986). It has been shown in a number of plant species, including soybean and safflower, that germinating seeds actively synthesize TAGs (Ichihara and Noda, 1981; Kwanyuan and Wilson, 1986; Wilson and Kwanyuan, 1986). The relatively high level of expression in roots is also consistent with the fact that root plastids are capable of synthesizing large amounts of triacylglycerol (Sparace et al., 1992).

Southern blot hybridization (Southern, 1975) was performed with genomic DNA digested with several restriction enzymes including BglII, EcoRI and HindIII. The *TAG1* gene has no internal BglII and HindIII site, while one internal EcoRI site exists. Our Southern analysis suggested that *TAG1* most likely represents a single copy gene in the *Arabidopsis* genome (Figure 6b).

An insertion mutation is found in the TAG1 gene in mutant AS11

Alignment of the genomic sequence (accession no. AC003058; SEQ ID NO:3) with that of the *TAG1* cDNA [SEQ ID NO:1] revealed that the *TAG1* gene contains 16 exons and 15 introns, spanning a region of about 3.4 kb (Figure 7a). DNA containing the *TAG1* allele from AS11 was PCR-amplified and completely sequenced. The AS11 *TAG1* allele has a 147-bp insertion located at the central region of intron 2. The insertion is a duplication of a segment that is composed of 12 bp from the 3' end of intron 1, the entire sequence of exon 2 (81 bp) and 54 bp from the 5' end of intron 2 (Figure 7a).

In order to rule out the possibility of PCR artifacts, two sets of primers were used to perform further PCR amplifications. Primers A and B (see Experimental Procedures, *Primer Strategy*) located in exons 1 and 3, respectively, amplified a DNA fragment that is about 150 bp longer from AS11 (Figure 7b, lane 2) than that from the wild type (Figure 7b, lane 1). The second pair of primers, C and B (Experimental Procedures), with one to be found in both exon 2 and the insertion segment, and the other located in exon 3, generated two amplified fragments from AS11 (Figure 7b, lane 4), while only one from the wild type (Figure 7b, lane 3). Hence these results confirmed that the insertion mutation the inventors identified

through sequencing, reflected the true nature of the mutation in the *TAG1* gene in the AS11 genome.

The AS11 TAG1 transcript has an 81-bp insertion in its open reading frame

Northern blot analyses indicated that there was no difference in the expression profiles of the *TAG1* gene, between the AS11 mutant and wild type *A. thaliana*. In order to investigate the effect of the mutation at the transcript level, reverse-transcription PCR (RT-PCR) was performed to amplify the *TAG1* transcript from RNA extracted from germinating seedlings of mutant AS11. Sequencing analysis revealed that there is an 81-bp insertion composed entirely of exon 2 in the transcript from AS11. The exon 2 in the repeat is properly spliced. The alteration of the transcript thus does not disturb the reading frame. However, this additional exon 2 sequence in the AS11 transcript would result in an altered DGAT protein with the 27 amino acid insertion ^{131}SHAGLFNLCVVVLIAVNSRLIIENLMK^{157} [SEQ ID NO:11]. The inventors' data shows that this insertion results in a 40-70% reduction in DGAT activity throughout seed development (Katavic et al., 1995). The 81 bp insert responsible for reduced DGAT activity in AS11 is visible in the comparison of RT-PCR products (Figure 7b: Compare lane 5 (WT) and lane 6 (AS11).) The DNA aberration observed in the AS11 mutant was unexpected, since ethyl methanesulfonate (EMS) generally causes point mutations. Although we cannot rule out the possibility that this AS11 mutant was the result of a spontaneous mutation event, EMS-induced deletions and insertions have been reported in other systems (Mogami *et al.*, 1986, Okagaki *et al.*, 1991)

The TAG1 gene insertion in Arabidopsis mutant AS11 affects seed triacylglycerol accumulation, but not sterol ester accumulation in seeds.

Because TAG1 also showed some sequence homology to acyl CoA: cholesterol acyltransferases (ACATs) from a number of species (Chang *et al.*, 1997), the inventors compared both triacylglycerol and sterol ester accumulation in seeds of the wild-type *A. thaliana* and AS11 mutant. While the triacylglycerol content and TAG/DAG ratios were reduced in AS11 (i.e. increased proportion of seed oil DAGs,) in contrast, the proportions of sterol esters in WT and AS11 seeds were similar, at 0.8 and 1% of the total lipid extract, respectively (Table 1). If the

TAG1 lesion affected ACAT-like activity, one might expect a reduction in seed sterol esters, but this was not observed. These results indicated that TAG1 is not involved in sterol-ester homeogenesis, and thus not an acyl CoA: sterol acyltransferase.

TAG1 expression in yeast.

The *TAG1* cDNA overexpressed in yeast resulted in a 3.5 to 4-fold increase in microsomal DGAT activity compared to plasmid only (*pYES2*) control transformants (Fig. 8), confirming that the *TAG1* gene product functions as a DGAT. When ^{14}C18:1-CoA was added to the yeast lysates, sterol esters were also labeled *in vitro* (data not shown), but there was no significant difference in the ^{14}C-labeled sterol esters produced by lysates from the *pYES2* GAL-induced control and the *pYES2:TAG1* Gal-induced transformant. This confirms that the TAG1 product does not encode an acyl-CoA: sterol acyltransferase (like ACAT).

Complementation of the A. thaliana AS11 Mutant Line by Transformation with the DGAT cDNA.

The cloned full-length DGAT cDNA was used as a template for PCR amplification with the primers DGATXbaI (CTAGTCTAGAATGGCGATTTTGGA; SEQ IN NO: 12) and DGATXhoI (GCGCTCGAGTTTCATGACATCGA; SEQ ID NO:13) to provide new restriction sites on each end of the sequence as described in Experimental Procedures. A 1.6kb fragment was excised by a XbaI/KpnI digestion and ligated into the corresponding sites of the pSE129 vector (provided by Dr. P. Covello, PBI/NRC). pSE129A is a vector derived from the plant transformation vector pRD400 (Datla et al. 1992). The vector pSE129A contains the seed-specific napin promoter and the nos terminator cloned into the EcoRI and HindIII sites of the pRD400 plasmid (Figure 9). Hence in the DGAT-pSE129A construct, the *Arabidopsis* DGAT cDNA is under the control of the napin promoter. The construct integrity was confirmed by sequencing.

The pSE129A containing the napin:DGATcDNA was introduced into *A. tumefaciens*, used to transform *A. thaliana* mutant AS11, and progeny analyzed as described in Experimental Procedures. A number of T_2 transgenic lines were isolated which complemented the fatty acid mutant phenotype found in AS11 (reduced 20:1 and elevated polyunsaturated C_{18}s), restoring the wild-type seed fatty

acid profile (Figure 10). This finding confirms the nature of the lesion in AS11 and directly ties the AS11 lipid phenotype to this mutation.

Over-Expression of the DGAT cDNA in Wild-Type A. thaliana

The cloned full-length DGAT cDNA was used as a template for PCR amplification with the primers DGATXbaI (CTAGTCTAGAATGGCGATTTTGGA ; SEQ ID NO:12) and DGATXhoI (GCGCTCGAGTTTCATGACATCGA; SEQ ID NO:13) to provide new restriction sites on each end of the sequence as described in Experimental Procedures. A 1.6kb fragment was excised by a XbaI/KpnI digestion and ligated into the corresponding sites of the pSE129 vector (provided by Dr. P. Covello, PBI/NRC). pSE129A is a vector derived from the plant transformation vector pRD400 (Datla et al. 1992). The vector pSE129A contains the seed-specific napin promoter and the nos terminator cloned into the EcoRI and HindIII sites of the pRD400 plasmid (Figure 9). Hence in the DGAT-pSE129A construct, the *Arabidopsis* DGAT cDNA is under the control of the napin promoter. The construct integrity was confirmed by sequencing.

The pSE129A containing the napin:DGATcDNA was introduced into *A. tumefaciens,* used to transform wild-type *A. thaliana*, and progeny analyzed as described in Experimental Procedures. A number of T_2 transgenic lines were isolated which exhibited an increased oil content (Figure 11) an increased average 100-seed weight (Figure 12) and a strong linear correlation between the two traits (Figure 13).

In terms of fatty acyl composition, wild type lines contaning over-expressed DGAT cDNA showed a decrease in the total saturates, and increases in the monounsaturates and in the 18:1/[18:2 + 18:3] index, as shown in Table 2 below. Such changes are all towards a 'healthier" oil profile, and can be applied directly to canola, other oilseeds in the *Brassicaceae* and other edible oil crops to produce similar oil composition improvements.

Table 2

Fatty acid composition of seed oil from *A. thaliana* non-transformed wild-type controls (WT Con) and three T2 transgenic lines (2', 9 and 11) of wild type transformed with the DGAT cDNA under the control of a napin promoter (napin: DGAT).

Line	Total Saturates[a] Wt %	Monounsaturates[b] Wt %	18:1/[18:2 + 18:3] index[c]
WT Control	15.1 ± 0.1	36.7 ± 0.2	29.9 ± 0.6
2' napin:DGAT	13.4	38.6	34.1
9 napin:DGAT	13.1	39.3	35.6
11 napin:DGAT	13.1	38.3	33.0

[a] Includes 16:0, 18:0, 20:0, 24:0

[b] includes 18:1, 20:1, 22:1, 24:1

[c] ([Wt % 18:1] + [Wt % 18:2 + Wt % 18:3]) x 100

EXPERIMENTAL PROCEDURES

Plant Material

Arabidopsis thaliana ecotype Columbia and mutant AS11 were grown under conditions described previously (Katavic et al., 1995). The A. thaliana mutant line AS11 was generated and characterized relative to wild type (WT) A. thaliana ecotype Columbia, as described by Katavic et al., (1995); (ATCC NO: PTA-1013).

DNA manipulation

Standard methods and procedures were used for DNA preparation, plasmid propagation and isolation (Sambrook *et al.*, 1989). Sequencing was conducted on an Applied Biosystems Model 373A DNA Sequencing System using the *Taq* DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). The nucleotide and the deduced amino acid sequences were compared with sequences available in databanks using the BLAST program (Altschul *et al.*, 1990).

Southern and Northern analysis

Total RNA was extracted from different tissues at various developmental stages, using the method of Lindstrom and Vodkin (1991). RNA samples were denatured with formaldehyde and separated on 1.2% formaldehyde-agarose gels. About 5 μg of total RNA was loaded, and the amount of RNA per lane was calibrated by the ethidium bromide-staining intensity of the rRNA bands. Genomic DNA was isolated, digested with restriction enzymes and a Southern blot analysis was performed according to Sambrook et al. (1989). The *TAG1* DNA probe was ^{32}P labeled by random-priming according to protocols of the manufacture (BRL).

PCR strategy

Primers used for the amplification of the *TAG1* gene were as follows: DGAT1 (AGACACGAATCCCATTCCCACCGA; SEQ ID NO:14), DGAT2 (AGTGGTGACAACGCAGGGATGATG; SEQ ID NO:15), DGAT3 (ATGGTCGCTCCCACATTGTGT; SEQ ID NO:16), DGAT4 (CATACAATCCCCATGACATTTATCA; SEQ ID NO:17). DGAT1 and DGAT2 amplify the 5' half of the *TAG1* gene and DGAT3 and DGAT4 amplify the 3' end of the *TAG1* gene. Genomic DNA from AS11 was used as template for PCR amplification of the mutant *TAG1* allele using the thermal profile: 94° C 3 min; 40 cycles of 94° C 30 seconds, 62° C 45 seconds, 72° C 1 min; and 72° C 15 min. To further confirm the mutation, primers A (CGACCGTCGGTTCCAGCTCATCGG: [SEQ ID NO:18]) and B (GCGGCCAATCTCGCAGCGATCTTG; [SEQ ID NO:19]), as well as primers C (TAAACAGTAGACTCATCATCG; [SEQ ID NO:20]) and B, were used in pairs, respectively, to amplify the internal fragment containing the mutation. The primers DGAT1 and DGAT4 were used for PCR amplification of the cDNA with an *A. thaliana* silique cDNA library as template. Primers A and B were also used in RT- PCR amplification of the cDNA fragment encompassing the insertion segment.

Construction of TAG1 Multicopy Vector and Transformation and Characterization of DGAT Expression in Yeast

The TAG1 cDNA was cloned into pBluescript SK as described (Hadjeb and Berkowitz, 1996). The cDNA was cut out from the vector with KpnI/XbaI, and subsquently cloned into the respective sites of the yeast expression vector pYes2 (Invitrogen). The construct was confirmed by sequencing. Constructs with TAG1

transcription under the control of the GAL1 promoter released a fragment of approximately 1.9 kb. Because the TAG1 fragment has its own initiating ATG codon, the product expressed is not a fusion protein. As a host for yeast expression, an SLC deletion strain (YMN5 [slc1Δ2::LEU2 ura3]) (kindly provided by M.M. Nagiec and R.C. Dickson, University of Kentucky, Lexington, KY; Nagiec et al., 1993) was used; we reasoned that in this mutant, the endogenous DAG pool may be lower than in WT yeast, and that this would allow us to maximize the activity from over-expressed TAG1 in the presence of exogenously supplied ^{14}C-DAG during in vitro DGAT assays of transformant lysates. Yeast transformation was performed according to Elble (1992). YMN5 transformants containing vector only (pYES2) were used as controls. Single colonies were cultured overnight in 20 mL of SD medium (Synthetic Dextrose medium with glucose and without uracil, as described by Ausubel et al., 1995, Vol. 2, p. 13.1.3) on a rotary shaker (270 rpm) at 28°C. Cells were pelleted from the overnight culture and resuspended in 50 mL of medium for induction of expression (SD medium containing galactose and without uracil). Cells were reincubated at 28°C, with shaking at 270 rpm, and harvested after 4-6 hr. GAL-induced yeast transformants were harvested by centrifugation at 5000 rpm for 5 min and resuspended in 100 mM Hepes-NaOH, pH 7.4, containing 1 mM EDTA and 1 mM DTT. Cell lysates were prepared using acid-washed glass beads as described by Ausubel et al. (1995). Protein in yeast lysates was measured using the Bradford (1976) assay, protein levels in each lysate were normalized and aliquots (250 μg protein) were assayed for DGAT activity as described below.

Lipid Substrates and DGAT Analyses

^{14}C-labeled diolein [1-^{14}C oleic] (Sp. activity 55 mCi/ mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). The ^{14}C-labelled *sn*-1,2-diolein isomer was purified by TLC on borate-impregnated plates and emulsified in Hepes buffer the presence of 0.2% Tween-20 as described by Taylor et al., (1991). 20:1-CoA, CoASH, ATP, and all other biochemicals were purchased from Sigma.

DGAT assays were conducted at pH 7.4, with shaking at 100 rpm in a water bath at 30°C for 30-60 min. Assay mixtures (0.5 mL final volume) contained lysate protein (250 μg), 90 mM Hepes-NaOH, 0.5 mM ATP, 0.5 mM CoASH, 1 mM $MgCl_2$, 200 μM *sn*-1,2 diolein (sp. activity 2 nC/nmol) in 0.02% Tween 20, and 18 μM 20:1-

CoA as the acyl donor. The ^{14}C-labeled TAGs were isolated by TLC and quantified as described by Taylor et al (1991).

Further Lipid and Sterol Ester Analyses in AS11 and WT:

Total lipid extracts (TLEs), and lipid class analyses in WT and the AS11 mutant were performed as described by Taylor *et al.*, (1991; 1992) and by Katavic *et al.*, (1995). Relative seed oil content was also measured by magic angle sample spinning ^{1}H-NMR, according to the method of Rutar (1989). Analyses were conducted with 200-seed samples of intact wild-type and AS11 seeds using a Bruker AM wide-bore spectrometer (Bruker Analytische Masstechnik GHBH, Silberstreifen D-76287, Rheinstetten4/Karlstuhe, Germany) operating at 360 MHz. To reduce anisotropic line broadening, the seed sample was rotated at 1 kHz in a zirconium rotor oriented 54.7° to the magnetic field. The integration response for resonances attributable to liquid-like oil were summed and the value for AS11 seed was recorded relative to the response for the WT control seed sample, the latter set at a value of 1.00.

Sterol esters were purified from the TLEs by thin layer chromatography (TLC) on Silica H plates developed in hexane: diethyl ether: formic acid (80:20:2, v/v/v). After elution from the silica H with chloroform: methanol (2:1, v/v), the sterol esters were quantified by saponification followed by methylation of the resulting fatty acids with 3N methanolic-HCl. The fatty acid methyl esters (FAMEs) were analyzed by GC as described previously (Taylor et al., 1991). The free sterols released by saponification were also analyzed by GC on a 30 m DB-5 column; GC temperature program : initial temp: 180°C, increasing at 10°C/min to 300°C and held at this temperature for 15 min. The sterol ester content was reported as a % of the TLE; i.e. FAMEs released from sterol esters calculated as proportion of the FAMEs released by transmethylation of the total lipid extract (TLE).

Construction of Plant Transformation Vector Containing the Wild-Type DGAT gene for Over-Expression in WT A. thaliana and Complementation of the A. thaliana AS11 Mutant:

Two primers:

Gen 1 (GAGAGGATCCACGCTCACGACCCATTCTTCCCG; [SEQ ID NO:21]), and

Gen 2 (AAGAAGGATCCATCCCCAAAACGGGACCACCAA; [SEQ ID NO:22])

were synthesized according to sequences upstream and downstream of the TAG1 gene. These primers were used to PCR amplify a genomic fragment of 5.1 kb from wild-type A. thaliana. The PCR fragment was purified and digested with BamHI and inserted into the corresponding site in plasmid pRD400 (Datla et al. 1992) to generate the plant transformation vector DGATg-pRD400.

Construction of DGAT cDNA Plant Transformation Vector for Seed-Specific Expression:

The cloned full-length DGAT cDNA was used as a template for PCR amplification with the primers DGATXbaI (CTAGTCTAGAATGGCGATTTTGGA; SEQ ID NO:12) and DGATXhoI (GCGCTCGAGTTTCATGACATCGA; SEQ ID NO: 13) to provide new restriction sites on each end of the sequence. The PCR profile was as follows: 94°C 1 min; 30 cycles of 94°C 30 seconds, 55°C 30 seconds, 72°C 1 min; and 72°C 5 min. The PCR product was then ligated into the PCR-2.1 vector (InVitrogen). A 1.6kb fragment was excised by a XbaI/KpnI digestion and ligated into the corresponding sites of the pSE129 vector (provided by Dr. P. Covello, PBI/NRC). pSE129A is a vector derived from the plant transformation vector pRD400 (Datla et al. 1992). The vector pSE129A contains the seed-specific napin promoter and the nos terminator cloned into the EcoRI and HindIII sites of the pRD400 plasmid (See Figure 9). Hence in the DGAT-pSE129A construct, the *Arabidopsis* DGAT cDNA is under the control of the napin promoter. The construct integrity was confirmed by sequencing.

Transformation of Agrobacterium with Plant DGAT Vector Constructs:

Electrocompetent *Agrobacterium* cells, GV3101 (pMP90) strain, were prepared as follows: An *Agrobacterium* culture was grown 24 to 48 hrs in 2YT, and when the absorbance at 600 nm reached 0.5 to 0.7, the cells were chilled on ice and pelleted by centrifugation (5,000 *x g*, 10 min in a GSA rotor at 4°C). The pellet was washed in 1, 0.5 and 0.02 volumes of cold 10% sterile glycerol and

resuspended in 0.01 volume of cold 10% glycerol. The electrocompetent cells were then frozen in liquid N_2 and stored at -70°C. The *Agrobacterium* cells were transformed by electroporation with 20-50 ng of transforming DNA (either DGATg-pRD400 or DGAT-pSE129A) according to the manufacturer's instructions, plated on a selective medium (LB with 50 µg/mL kanamycin) and incubated overnight at 28°C. Single transformed cells were grown overnight (28°C, 225 r.p.m.) in 5 mL LB with 50 µg/mL Kanamycin and 25 µg/mL Gentamycin. DNA extraction and purification were performed with a Qiaprep Spin Miniprep kit (Qiagen). The fidelity of the construct was re-checked by DNA sequencing before plant transformation.

Transformation of Arabidopsis thaliana:

The transformation protocol was adapted from that described by Clough and Bent (1998). Seeds of *Arabidopsis thaliana* ecotype Columbia and mutant AS11 (Katavic *et al.*, 1995) were grown at 22°C under fluorescent illumination (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) in a 16 h light/8 hour dark regime. Typically, four to six plants were raised in a 10 cm^2 pot in moistened Terra-lite Redi-earth (W. R. Grace & Co. Canada Ltd. Ajax, ON, Canada). To prevent the soil mix in the pot from falling into the inoculation media, soil was mounded as a platform with seeds sown on top, and the whole pot covered by a nylon window screen and secured by a rubber band. Plants were vacuum infiltrated in an *Agrobacterium* suspension when the first flowers started opening.

To grow *Agrobacterium*, a 5 mL suspension in LB medium containing 50 µg/mL kanamycin and 25 µg/mL gentamycin was cultured overnight at 28°C. The day before infiltration, this "seed culture" was divided into four flasks containing 250 mL of LB medium supplemented with 50 µg/mL kanamycin and 25 µg/mL gentamycin. These culture were grown overnight at 28°C. The next morning after the absorbance at 600 nm was checked (approximately = 1.0), the cells were harvested by centrifugation (5,000 *x g*, 10 min in a GSA rotor at room temperature) and resuspended in the infiltration medium (sucrose 5%; Silwet-77 0.005% in water) to obtain an optical density at 600 nm of 0.8. The *Agrobacterium* suspension was then poured into a beaker and the potted plants inverted into the beaker so that the flowers and bolts were submerged. The beaker was then placed into a large Bell jar and a vacuum drawn using a vacuum pump, until bubbles formed on the stem

surfaces and the solution started to bubble slightly, and then the vacuum was released rapidly. [*Note:* The necessary time and pressure will vary from one lab setup to the next, but good infiltration is visibly apparent as uniformly darkened, water-soaked tissue.] Pots were removed from the beaker, laid on their side in a plastic tray and covered with a plastic dome, to maintain humidity. The following day, the plants were uncovered, set upright and allowed to grow for approximately four weeks in a growth chamber under continuous light conditions as described by Katavic et al., (1995). When the siliques were mature and dry, seeds were harvested and selected for positive transformants.

Selection of Putative Transformants (Transgenic plants) and Analysis of Transgenic Plants:

For each construct, seeds were harvested in bulk. Seeds were surface-sterilized by submerging them in a solution containing 20% bleach and 0.01% Triton X-100 for 20 min, followed by three rinses with sterile water. Sterilized seeds were then plated by resuspending them in sterile 0.1% phytagar at room temperature (about 1 mL phytagar for every 500-1000 seeds), and then applying a volume containing 2,000-4,000 seeds onto 150 x 15 mm kanamycin selection plate. Plates were incubated for 2 days in the cold without light, and then grown for 7-10 days in a controlled environment (22°C under fluorescent illumination (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) in a 16 h light/8 hour dark regime). The selection media contain ½ MSG medium, 0.8% phytagar, 3% sucrose, 50ug/mL kanamycin and 50ug/ mL Timentin. Petri dishes and lids were sealed with a Micropore™ surgical tape tape (3M Canada, London, ON, Canada). After 7-10 days, drug-resistant plants that had green leaves and well established roots within the medium were identified as transformants and at the 3-5 leaf stage, selected transformants were transplanted into flats filled with heavily moistened soil mix. Transformants were grown to maturity and mature seeds (T_2 generation as defined in Katavic et al., (1994)) were harvested from individualplants, and further analyzed.

DNA isolation from and analysis of Transformants

Genomic DNA was isolated from individual T_1 plants following the protocol of Dellaporta *et al.*, (1983). A PCR amplification using the paired primers described previously for the DGAT cDNA or for the DGAT gene, was performed to confirm the

presence of the cDNA or the gene, respectively, in the T_1 transformants. Southern analyses (Southern, 1975) were performed to select the transformants containing a single copy of the inserted fragment. DNA samples were digested with restriction enzymes (Bgl II for the DGAT cDNA and Eco RI for the DGAT gene), resolved by electrophoresis on a 1% agarose gel, and Southern blotting performed using a nylon filter (Hybond-N+, Amersham) according to Sambrook *et al.* (1989). The DGAT cDNA fragment, labelled with α–[^{32}P] dCTP (NEN/DuPont) using the Random Primer DNA labelling kit (Gibco BRL), was used as a probe. Hybridization was performed at 60°C according to Church and Gilbert (1984). The filter was then exposed to Kodak X-OMAT-AR film.

DEPOSIT INFORMATION

The following biological material has been deposited at the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Virginia, 20110-2209, U.S.A. All of these deposits were made on behalf of the Applicant/Assignee (National Research Council of Canada) under the terms of the Budapest Treaty on the dates indicated, and have been given the accession numbers shown below.

Deposited Material	Date of Deposit	Accession No.
Arabidopsis DGAT gene	November 29, 1999	PTA-988
Arabidopsis DGAT cDNA	November 29, 1999	PTA-989
Arabidopsis ASII seeds	December 3, 1999	PTA-1013

The deposit receipts are shown later in this description.

SEQUENCE LISTING FREE TEXT

The Sequence Listing provided below contains free text entries in respect of SEQ ID NOs: 12 to 22. The free text used in the Sequence Listing is repeated as follows:

SEQ ID NO:12	Primer of DGATXbal
SEQ ID NO:13	Primer of DGATXhol

SEQ ID NO:14	Primer DGAT1
SEQ ID NO:15	Primer DGAT2
SEQ ID NO:16	Primer DGAT3
SEQ ID NO:17	Primer DGAT 4
SEQ ID NO:18	Primer A
SEQ ID NO:19	Primer B
SEQ ID NO:20	Primer C
SEQ ID NO:21	Primer Gen 1
SEQ ID NO:22	Primer Gen 2.

A summary of all of the listed sequences is provided below for ease of review:

SEQ ID NO:1 - (pDGAT; vector containing isolated and purified deoxyribonucleic acid cDNA; ATCC No PTA-989), Genbank/EMBL Accession No. AJ238008.

SEQ ID NO:2 - The deduced amino acid sequence of the *Arabidopsis* DGAT (AtTAG1) protein.

SEQ ID NO:3 - (pgenomic DGAT; vector containing isolated and purified genomic deoxyribonucleic acid (genomic DNA) ATCC No PTA-988).

SEQ ID NO:4 - MDGAT, mouse DGAT [GenBank/EMBL Accession No. AF078752 (Cases *et al.*, 1998)].

SEQ ID NO:5 - HARGP1, human ARGP1 protein [GenBank/EMBL Accession No. AF059202; Oelkers et al., 1998].

SEQ NO:6 - *Arabidopsis thaliana* expressed sequence tag (EST) [accession no. AA042298).

SEQ ID NO:7 - A diacylglycerol/phorbol ester-binding motif found in SEQ ID NO:2, SEQ ID NO:8 and SEQ ID NO:9 (^{414}HKWMVRHIYFP^{424}).

SEQ ID NO:8 - *B. napus* DGAT amino acid sequence GenBank EMBL Accession No AF155224.

SEQ ID NO:9 - *B. napus* DGAT amino acid sequence GenBank/EMBL Accession No. AF164434.

SEQ ID NO: 10 - Targeting motif typical of members of the SnRK1 protein kinase family found in SEQ ID NO:2, SEQ ID NO:8 and SEQ ID NO:9 X-L^{200}-X-K^{202}-X-X-S^{205}-X-X-X-V^{209}

SEQ ID NO:11 - A 27 amino acid insertion repeat in SEQ ID NO:2 found in the *Arabidopsis thaliana* AS11 mutant.

^{131}SHAGLFNLCVVVLIAVNSRLIIENLMK^{157}

SEQ IN NO: 12 - CTAGTCTAGAATGGCGATTTTGGA (nucleotide sequence of Primer DGATXbal).

SEQ ID NO:13 - GCGCTCGAGTTTCATGACATCGA (nucleotide sequence of Primer DGATXhol).

SEQ ID NO:14 - AGACACGAATCCCATTCCCACCGA (nucleotide sequence of Primer DGAT1).

SEQ ID NO:15 - AGTGGTGACAACGCAGGGATGATG (nucleotide sequence of Primer DGAT2).

SEQ ID NO:16 - ATGGTCGCTCCCACATTGTGT (nucleotide sequence of Primer DGAT3).

SEQ ID NO:17 - CATACAATCCCCATGACATTTATCA (nucleotide sequence of Primer DGAT4).

SEQ ID NO:18 - CGACCGTCGGTTCCAGCTCATCGG (nucleotide sequence of Primer A).

SEQ ID NO:19 - GCGGCCAATCTCGCAGCGATCTTG (nucleotide sequence of Primer B).

SEQ ID NO:20 - TAAACAGTAGACTCATCATCG (nucleotide sequence of Primer C).

SEQ ID NO:21 - GAGAGGATCCACGCTCACGACCCATTCTTCCCG (nucleotide sequence of primer Gen 1).

SEQ ID NO:22 - AAGAAGGATCCATCCCCAAAACGGGACCACCAA

(nucleotide sequence of primer Gen 2).

SEQ ID NO:23 - AS11 mutant DGAT cDNA nucleotide sequence.

SEQ ID NO:24 - AS11 mutant DGAT genomic DNA nucleotide sequence.

SEQ ID NO:25 - the deduced amino acid sequence of SEQ ID NO:23.

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

NRC Canada
Attn: David C. Taylor
Plant Biotechnology Institute
110 Gymnasium Place
Saskatoon, SK S7N 0W9 Canada

Deposited on Behalf of: National Research Council of Canada

Identification Reference by Depositor:	**Patent Deposit Designation**
Arabidopsis DGAT gene	PTA-988
Arabidopsis DGAT cDNA	PTA-989

The deposits were accompanied by: __ a scientific description X a proposed taxonomic description indicated above. The deposits were received November 29, 1999 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will not inform you of requests for the strains.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested December 2, 1999 On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara M. Halley

Date: December 3, 1999

Barbara M. Halley, Administrator, Patent Depository

cc: Kirby, Eades, Gale and Baker

BP/9

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

National Research Council of Canada
Attn: David C. Taylor
Plant Biotechnology Institute
110 Gymnasium Place
Saskatoon, SK S7N OW9
Canada

Deposited on Behalf of: National Research Council of Canada

Identification Reference by Depositor: *Arabidopsis* AS11

Patent Deposit Designation: PTA-1013

The seeds were accompanied by: _ a scientific description X a proposed taxonomic description indicated above.
The seeds were received December 3, 1999 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will not inform you of requests for the seeds..

The seeds will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the seeds and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said seeds.

If the seeds should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with viable seeds of the same.

The seeds will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the seeds cited above was tested December 23, 1999. On that date, the seeds were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Depository

Date: January 3, 2000

cc: Kirby, Eades et al.

REFERENCES RELEVANT TO THE CURRENT INVENTION

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J (1990) Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Stuhl, K., eds (1995). Current Protocols in Molecular Biology, Vols 1, 2, and 3. Wiley, New York.

Bechtold, N., Ellis, J., and Pelletier, G. (1993) *In planta Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C R Acad Sci Paris, Sciences de la vie/Life sciences* 316: 1194-1199.

Becker, D., Brettschneider, R. and Lörz, H. (1994) Fertile transgenic wheat from microprojectile bombardment of scutellar tissue. *Plant J.* 5: 299-307.

Bernerth R, Frentzen M (1990) Utilization of erucoyl-CoA by acyltransferases from developing seeds of *Brassica napus* (L.) involved in triacylglycerol biosynthesis. *Plant Sci* 67: 21-28.

Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.

Budziszewski, G.J., Croft, K.P.C. and Hildebrand, D.F. (1996) Uses of biotechnology in modifying plant lipids. *Lipids* 31: 557-569.

Cao, Y-Z and Huang, AHC (1986) Diacylglycerol acyltransferase in maturing oil seeds of maize and other species. *Plant Physiol.* 82: 813-820.

Cao Y-Z, Huang AHC (1987) Acyle coenzyme A preference of diacylglycerol acyltransferase from maturing seeds of *Cuphea*, maize, rapeseed and canola. *Plant Physiol.* 84: 762-765

Cases, S., Smith, J.S., Zheng, Y-W., Myers, H.M., Lear, S.R., Sande, E., Novak, S., Collins, C., Welch, C.B., Lusis, A.J., Erickson, S.K. and Farese, R.V., JR. (1998) Identification of a gene encoding a acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Nat'l. Acad. Sci. USA*, 95, 13018-13023.

Chang, T.Y., Chang, C.C.Y. and Cheng, D. (1997) Acyl-Coenzyme A: Cholesterol Acyltransferase. *Annu. Rev. Biochem.* 66, 613-38.

Church, G.M. and Gilbert, W. (1984) Genomic sequencing. Proc. Natl. Acad. Sci. USA. 81, 1991-95.

Clough, S.J. and Bent, A.F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. The Plant Journal 16, 735-43

Datla, R.S.S., Hammerlindl, J.K., Panchuk, B., Pelcher, L.E. and Keller, W.A. (1992) Modified binary plant transformation vectors with the wild-type gene encoding NPTII. *Gene* 211: 383-384.

Datla, R.S.S., Bekkaoui, F., Hammerlindl, J., Pilate, G., Dunstan, D.I. and Crosby, W.L. (1993) Improved high-level constitutive foreign gene expression in plants using an AMV RNA4 untranslated leader sequence. *Plant Sci.* 94: 139-149.

Datla, R., Anderson, J.W. and Selvaraj, G. (1997) Plant promoters for transgene expression. *Biotechnology Annual Review* 3: 269-296.

DeBlock, M., DeBrouwer, D. and Tenning P. (1989) Transformation of *Brassica napus* and *Brassica oleracea* using *Agrobacterium tumefaciens* and the expression of the *bar* and *neo* genes in the transgenic plants. *Plant Physiol.* 91: 694-701.

De Lange, P., Van Blokland, R., Kooter, J.M. and Mol, J.M.N. (1995) Suppression of flavenoid flower pigmentation genes in *Petunia hybrida* by the introduction of antisense and sense genes. *In:* Gene Silencing in Higher Plants and Related Phenomena in Other Eukaryotes. P.Meyer (Ed.), Springer-Verlag, Berlin, pp. 55-75.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983) A plant DNA minipreparation:Version II. Plant Mol. Biol. Rep. 1, 19-21

Elble, R. (1992). A simple and efficient procedure for transformation of yeasts. *Biotechniques* 13,18-20.

Focks, N. and Benning, C. (1998) *wrinkled1*: A novel, low-seed-oil mutant of Arabidopsis with a deficiency in the seed-specific regulation of carbohydrate metabolism. *Plant Physiol.* 118, 91-101.

Frentzen M (1993) Acyltransferases and triacylglycerols. *In:* Moore, Jr. TS, editor, *Lipid Metabolism in Plants*, pp. 195-230. CRC Press, Ann Arbor,.

Giraudat, J., Hauge, B.M., Valon, C., Smalle, J., Parcy, F., Goodman, H.M. (1992) Isolation of the *Arabidopsis* ABI3 gene by positional cloning. *Plant Cell* 4, 1251-1261.

Goodman, H.M., Ecker, J.R. and Dean, C. (1995) The genome of *Arabidopsis thaliana*. *Proc. Nat'l. Acad. Sci. USA* 92: 10831-10835.

Hadjeb N and Berkowitz GA (1996) Preparation of T-over-hang vectors with high PCR product cloning efficiency. *Biotechniques* 20: 21-22.

Halford, N.G. and Hardie, D.G. (1998) SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Mol. Biol.* 37, 735-748.

Hitz, W.D., Mauvis, C.J., Ripp, K.G., Reiter, R.J., DeBonte, L. and Chen, Z. (1995) The use of cloned rapeseed genes for cytoplastic fatty acid desaturases and the plastid acyl-ACP thioesterases to alter relative levels of polyunsaturated and saturated fatty acids in rapeseed oil. Proc. 9th Internat'nal Cambridge Rapeseed Congress UK, pp. 470-472.

Ichihara, K. and Noda, M. (1981) Lipid synthesis in germinating safflower seeds and protoplasts. *Phytochemistry* 20, 1245-1249.

Ichihara, K., Takahashi, T. and Fujii, S. (1988) Diacylglycerol acyltransferase in maturing safflower seeds: its influences on the fatty acid composition of the triacylglycerol and on the rate of triacylglycerol synthesis. *Biochim. Biophys. Acta* 958, 125-129.

Jorgensen, R.A. and Napoli, C.A. (1994) Genetic engineering of novel plant phenotypes. U.S. Patent No. 5283184.

Josefsson, L-G, Lenman M, Ericson ML and Rask L (1987) Structure of a gene encoding the 1.7S storage protein, napin, from *Brassica napus*. *J biol Chem* 262: 12196-12201.

Katavic, V., Haughn, G.W., Reed, D., Martin, M. and Kunst, L. (1994) In planta transformation of Arabidopsis thaliana. Mol. Gen. Genet. 245: 363-370.

Katavic, V., Reed, D.W., Taylor, D.C., Giblin, E.M., Barton, D.L., Zou, J-T., MacKenzie, S.L., Covello, P.S. and Kunst, L. (1995) Alteration of fatty acid composition by an EMS-induced mutation in Arabidopsis thaliana affecting diacylglycerol acyltransferase activity. Plant Physiol. 108, 399-409.

Kinney, A.J. (1995) Improving soybean seed quality. In: Induced Mutations and Molecular Techniques for Crop Improvement. International Atomic Energy Agency, Vienna, Austria., pp. 101-113.

Kinney, A.J. (1997) Genetic engineering of oilseeds for desired traits. In: Genetic Engineering, Vol. 19 (J.K. Setlow, ed.), Plenum Press, NY., pp. 149-166.

Kishore G.M. and Somerville, C.R. (1993) Genetic engineering of commercially useful biosynthetic pathways in transgenic plants. Current Opinion in Biotechnology. 4: 152-158.

Knutzon, D.S., Thompson, G.A., Radke, S.E., Johnson, W.B., Knauf, V.C., and Kridl, J.C. (1992) Modification of Brassica seed oil by anti-sense expression of a stearoyl-acyl carrier protein desaturase gene. Proc. Nat'l Acad. Sci. USA, 89: 2624-2628.

Knutzon, D.S., Lardizabal, K.D., Nelson,J.S., Bleibaum, J.L., Davis, H.M and Metz, J. (1995) Cloning of a coconut endosperm cDNA encoding a 1-acyl-sn-glycerol-3-phosphate acyltransferase that accepts medium chain length substrates. Plant Physiol. 109, 999-1006.

Kwanyuen, P. and Wilson, R.F. (1986) Isolation and purification of diacylglycerol acyltransferase from germinating soybean cotyledons. Biochim. Biophys. Acta 877, 238-245.

Lacey DJ, Hills MJ (1996) Heterogeneity of the endoplasmic reticulum with respect to lipid synthesis in developing seeds of Brassica napus L. Planta 199: 545-551.

Lagercrantz, U., Putterill, J., Coupland, G. and Lydiate, D. (1996) Comparative mapping in Arabidopsis and Brassica, fine scale genome collinearity and congruence of genes controlling flowering. Plant J. 9: 13-20.

Lassner, M.W., Levering, C.K., Davis, H.M. and Knutzon, D.S. (1995) Lysophosphatidic acid acyltransferase from meadowfoam mediates insertion of erucic acid at the sn-2 position of triacylglycerol in transgenic rapeseed oil. Plant Physiol. 109, 1389-1394.

Lassner, M.W., Lardizabal, K, and Metz, J.G. (1996) A jojoba β-ketoacyl-CoA synthase cDNA complements the canola fatty acid elongation mutation in transgenic plants. The Plant Cell, 8: 281-292.

Lewin TM, Wang P and Coleman RA (1999) Analysis of amino acid motifs diagnostic for the sn-glycerol-3-phosphate acyltransferase reaction. Biochemistry 38: 5764-5771.

Lindstrom J.T. and Vodkin L.O. (1991) A soybean cell wall protein is affected by seed colour genotype. Plant Cell 3:561-571

Little D, Weselake RJ, Pomeroy MK, Furukawa-Stoffer T and Bagu J (1994) Solubilization and characterization of diacylglycerol acyltransferase from microspore-derived cultures of oilseed rape. Biochem J 304: 951-958.

Lloyd, A.M., Walbot, V. and Davis, R.W. (1992) Arabidopsis and Nicotiana anthocyanin production activated by maize regulators R and C1. Science 258: 1773-1775.

MacKenzie, S.L. and Jain, R.K. (1997) Improvement of oils crops via biotechnology. Recent Res. Dev. In Oil Chem. 1: 149-158.

Mayorek, N, Grinstein I, and Bar-Tana J (1989) Triacylglycerol synthesis in cultured rat hepatocytes. The rate-limiting role of diacylglycerol acyltransferase. Eur J Biochem 182: 395-400.

Meyer, P. (1995) Understanding and controlling transgene expression. Trends in Biotechnology, 13: 332-337.

Meyerowitz, E.M. (1987) Arabidopsis thaliana. Ann. Rev. Genet. 21: 93-111.

Meyerowitz, E.M. and Chang, C. (1985) Molecular biology of plant growth and development: Arabidopsis thaliana as an experimental system. In: Developmental Biology, Vol. 5, Plenum Press, NY., pp. 353-366.

Mogami, K., O'Donnell, P.T., Bernstein, S.I., Wright, T.R.F and Emerson, C.P., JR. (1986) Mutations of the Drosophila myosin heavy-chain gene: effects on transcription, myosin accumulation, and muscle function. Proc. Nat'l. Acad. Sci. USA. 83, 1393-1397.

Mol, J.M.N., Van der Krol, A.R., Van Tunen, A.J., Van Blokland, R., De Lange, P. and Stuitje, A.R. (1990) Regulation of plant gene expression by antisense RNA. FEBS Lett. 268: 427-430.

Moloney, M.M., Walker, J.M. and Sharma, K.K. (1989) High efficiency transformation of Brassica napus using Agrobacterium vectors. Plant Cell Rep. 8: 238-242.

Nagiec, M.M., Wells, G.B., Lester, R.L., and Dickson, R.C. (1993). A suppressor gene that enables Saccharomyces cerevisiae to grow without making sphingolipids encodes a protein that resembles an Escherichia coli fatty acyltransferase. J. Biol. Chem. 268, 22156-22163.

Nehra, N.S., Chibbar, R.N., Leung, N., Caswell, K., Mallard, C., Steinhauer, L. Baga, M. and Kartha K.K. (1994) Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. Plant J. 5: 285-297.

Nykiforuk C, Laroche A and Weselake RJ (1999) Isolation and sequence analysis of a novel cDNA encoding a putative diacylglycerol acyltransferase from a microspore-derived cell suspension culture of Brassica napus L. cv Jet Neuf (Accession No. AF155224).), Plant Physiology 120: PGR99-123.

Oelkers, P., Behar, A., Cromley, D., Billheimer, J.T. and Sturley, S.T. (1998) Characterization of two human genes encoding acyl Coenzyme A: cholesterol acyltransferase-related enzymes. J. Biol. Chem. 273, 26765-26771.

Okagaki, R.J., Neuffer, M.G. and Wessler, S.R. (1991) A deletion common to two independently derived Waxy mutations in maize. Genetics 128, 425-431.

Okuley, J., Lightner, J., Feldmann, K., Yadav, N., Lark, E. and Browse, J. (1994) Arabidopsis fad2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. The Plant Cell 6: 147-158.

Perry, H.Y. and Harwood, J.L. (1993a) Changes in the lipid content of developing seeds of Brassica napus. Phytochemistry 32: 1411-1415.

Perry, H.Y. and Harwood, J.L. (1993b) Use of [2-3H] glycerol precursor in radiolabelling studies of acyl lipids in developing seeds of Brassica napus. Phytochemistry 34: 69-73.

Poirier, Y., Dennis DE, Klomparens K and Somerville C (1992) Polyhydroxybutyrate, a biodegradable thermoplastic produced in transgenic plants. Science 256: 520-523.

Poirier, Y., Nawrath C and Somerville C (1995) Production of polyhydroxyalkanoates, a family of biodegradeable plastics and elastomers in bacteria and plants butyrate, a biodegradable thermoplastic produced in transgenic plants. Bio-Technology, 13: 142-150.

Poirier, Y., Ventre, G and Caldelari, D(1999) Increased flow of fatty acids toward β oxidation in developing seeds of Arabidopsis deficient in diacylglycerol acyltransferase activity or synthesizing medium-chain-length fatty acids. Plant Physiology 121: 1359-1366.

Potrykus, I. (1991) Gene transfer to plants: Assessment of published approaches and results. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 205-225.

Radke SE, Andrews BM, Moloney MM, Crouch ML, Kridl JC, and Knauf VC (1988) Transformation of Brassica napus L. using Agrobacterium tumefaciens: developmentally regulated expression of a reintroduced napin gene. Theor. Appl. Genet. 75: 685-694.

Rhodes, C.A., Pierce, D.A., Mettler, I.J., Mascarenhas, D. and Detmer, J.J. (1988) Genetically transformed maize plants from protoplasts. Science 240: 204-207.

Rutar, V. (1989) Magic angle sample spinning NMR spectroscopy of liquids as a non-destructive method for studies of plant seeds. J. Agric. Food. Chem. 37, 67-70.

Sambrook J., Fritsch E.F. and Maniatis T. (1989) In Molecular Cloning, A Laboratory Manual, 2nd edition. Cold Spring Harbor Laboratory Press.

Sanford, J.C., Klein, T.M., Wolf, E.D. and Allen, N. (1987) Delivery of substances into cells and tissues using a particle bombardment process. J. Part. Sci. Technol. 5: 27-37.

Settlage, SH, Wilson RF and Kwanyuen, P. (1995) Localization of diacylglycerol acyltransferase to oil body associated endoplasmic reticulum. Plant Physiol. Biochem. 33: 399-407.

Shimamoto, K., Terada, R., Izawa, T. and Fujimoto, H. (1989) Fertile transgenic rice plants regenerated from transformed protoplasts. Nature 338: 274-276.

Somerville, C.R. (1993) Future prospects for genetic modification of the composition of edible oils from higher plants. Am. J. Clin. Nutr. 58 (2 Suppl.): 270S-275S.

Songstad, D.D., Somers, D.A. and Griesbach, R.J. (1995) Advances in alternative DNA delivery techniques. Plant Cell, Tissue and Organ Culture 40: 1-15.

Southern E.M. (1975) Detection of specific sequences among DNA fragments separated by gele electrophoresis. J. Mol. Biol. 98: 503-517.

Sparace S.A., Kleppinger-Sparace K.F., Stahl R.J., Xue L. and Qi, Q. (1992) Lipid biosynthesis in pea root plastids and some effects of glycolytic intermediates. In SL MacKenzie and DC Taylor, eds. Seed Oils for the Future, AOCS Press, Champaign, IL. pp 52-60.

Stark, D.M., Timmerman, K.P., Barry, G.F., Preiss, J. and Kishore, G.M. (1992) Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. Science 258: 287-292.

Stobart AK, Stymne S, Höglund S. (1986) Safflower microsomes catalyse oil accumulation in vitro: A model system. Planta 169: 33-37.

Stymne, S. and Stobart, A.K. (1987) Triacylglycerol Biosynthesis. In Stumpf, P.K. ed, The Biochemistry of Plants, Academic Press, New York. 9, 175-214.

Taylor. CB. (1998) Comprehending cosuppression. The Plant Cell 9: 1245-1249.

Taylor, D.C., Weber, N., Barton, D.L., Underhill, E.W., Hogge, L.R., Weselake, R.J. and Pomeroy, M.K. (1991) Triacylglycerol bioassembly in microspore-derived embryos of Brassica napus L. cv. Reston. Plant Physiol. 97, 65-79.

Taylor, D.C., Barton, D.L., Rioux, K.P., MacKenzie, S.L., Reed, D.W., Underhill, E.W., Pomeroy, M.K. and Weber, N. (1992) Biosynthesis of acyl lipids containing very-long chain fatty acids in microspore-derived embryos of Brassica napus L. cv. Reston. Plant Physiol. 99, 1609-1618.

Tijburg, LB, Geelen, MJ and van Golde LM (1989) Rgulation of the biosynthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanloamine in the liver. Biochim Biophys Acta 1004: 1-19.

Tzen TC, Cao Y, Laurent P, Ratnayake C and Huang HC (1993) Lipids, proteins and structures of seed oil bodies from diverse species. Plant Physiol. 101: 267-276.

Vasil, I.K. (1994) Molecular improvement of cereals. Plant Mol. Biol. 25: 925-937.

Vaucheret, H., Béclin C, Elmayan T, Feuerbach, F., Godon C, Morel J-B, Mourrain, P., Palauqui, J-C and Vernhettes S (1998) Transgene-induced gene silencing in plants. The Plant Journal 16: 651-659.

Voelker, T.A., Worrell, A.C., Anderson, L., Bleibaum, J., Fan, C., Hawkins, D.J., Radke, S.E., and Davies, H.M. (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. Science 257: 72-74.

Voelker, T.A., Hayes, T.R., Cramner, A.M., Turner, J.C., and Davies, H.M. (1996) Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed. The Plant Journal 9: 229-241.

Vogel, G and Browse, J (1996) Cholinephosphotransferase and diacylglycerol acyltransferase: Substrate specificities at a key branch point in seed lipid metabolism. Plant Physiol. 110: 923-931.

Walden, R. and Wingender, R. (1995) Gene-transfer and plant regeneration techniques. Trends in Biotechnology 13: 324-331.

Weselake, R.J., Taylor, DC, Pomeroy, M.K., Lawson SL, and Underhill EW (1991) properties of diacylglycerol acyltransferase from microspore-derived embryos of Brassica napus L. Phtochemistry : 30: 3533-3538.

Weselake, R.J., Pomeroy, M.K., Furukawa, T.L., Golden, J.L., Little, D.B. and Laroche, A. (1993) Developmental profile of diacylglycerol acyltransferase in maturing seeds of oilseed rape and safflower and micro-spore-derived cultures of oilseed rape. Plant Physiol. 102, 565-571.

Wilson, R.F. and Kwanyuan P. (1986) Triacylglycerol synthesis and metabolism in germinating soybean cotyledons. Biochim. Biophys. Acta 877, 231-237.

Yang, H., Bard, M., Bruner, D.A., Gleeson, A., Deckelbaum, R.J., Aljinovic, G., Pohl, T.M., Rothstein, R. and Sturley, S.L. (1997) Sterol esterification in yeast: a two-gene process. Science 272, 1353-1356.

Yu, C., Kennedy, N.J., Chang, C.C.Y. and Rothblatt, J.A. (1996) Molecular cloning and characterization of two isoforms of Saccharomyces cerevisiae Acyl-CoA: Sterol Acyltransferase. J. Biol. Chem. 271, 24157-24163.

Zou, J-T., Katavic, V., Giblin, E.M., Barton, D.L., MacKenzie, S.L., Keller, W.A., Hu, X. and Taylor, D.C. (1997) Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. The Plant Cell 9: 909-923.

The teachings of the above references are hereby incorporated by reference.

CLAIMS:

1. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:1 or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

2. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:3 or a part of SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:3.

3. A vector for transformation of plant cells, characterized in that said vector contains a deoxyribonucleic acid sequence according to SEQ ID NO:1, or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

4. A vector for transformation of plant cells, characterized in that said vector contains a deoxyribonucleic acid sequence according to SEQ ID NO:3, or a part of SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:3.

5. A vector for transformation of plant cells, characterized in that said vector contains a deoxyribonucleic acid sequence according to SEQ ID NO:23, which is SEQ ID NO: 1 altered to contain an 81 bp insertion, such that the deduced amino acid sequence of the encoded protein contains the repeated sequence SHAGLFNLCVVVLIAVNSRLIIENLMK according to SED ID NO:25, where the spacing and identity of the underlined amino acids are identical or are replaced by conserved substitutions.

6. A vector according to claim 3 or claim 5, characterized in that said sequence is present in said vector in a sense orientation.

7. A vector according to claim 3, characterized in that said sequence is present in said vector in an anti-sense orientation.

8. Plasmid pDGATcDNA (ATCC PTA-989).

9. Plasmid pDGATgene (ATCC PTA-988).

10. A plant having a genome, characterized in that the genome contains an introduced nucleotide sequence of SEQ ID NO:1, or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

11. A plant seed having a genome, characterized in that said genome contains an introduced nucleotide sequence of SEQ ID NO:1, or a part of SEQ ID NO:1, or a sequence that is substantially homologous to SEQ ID NO:1.

12. A genetically transformed plant, characterized in that said genome has been transformed by a vector according to claim 3 or claim 4 or claim 5.

13. A genetically transformed plant seed, characterized in that said seed has been transformed by a vector according to claim 3 or claim 4 or claim 5.

14. A plant seed as claimed in Claim 11 or Claiim 13, characterized by exhibiting an altered seed oil content compared to an average of a statistically-significant number of seeds of genomically-unmodified plants of the same genotype grown in identical conditions at the same time.

15. A plant seed as claimed in Claim 11 or Claim13, characterized by exhibiting an altered diacylglycerol content in its seed oil compared to an average of a statistically-significant number of seeds of genomically-unmodified plants of the same genotype grown in identical conditions at the same time.

16. A plant seed as claimed in 11 or 13, characterized by exhibiting a seed oil with an altered fatty acyl composition compared to an average of a statistically-significant number of seeds a genomically-unmodified plantof the same genotype grown in identical conditions at the same time.

17. A plant as claimed in 10 or 12, characterized by exhibiting an enhanced biomass compared to an average of a statistically-significant number of genomically-unmodified plants of the same genotype grown in identical conditions at the same time.

18. A seed as claimed in 11 or 13, characterized by exhibiting an enhanced biomass compared to an average of a statistically-significant number of seeds of genomically-unmodified plants of the same genotype grown under identical conditions at the same time.

19. A method of producing transgenic plants by introducing a nucleotide sequence into a genome of said plant, characterized in that said nucleotide sequence introduced into said genome includes SEQ ID NO:1 or SEQ ID NO:3, or a part of SEQ ID NO:1 or SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:1, or to SEQ ID NO:3, or to or SEQ ID NO: 1, or a part of SEQ ID NO:1 or SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:1 containing an 81 bp insertion [SEQ ID NO:23] such that the deduced amino acid sequence of the encoded protein contains the repeated sequence SHAGLFNLCVVVLIAVNSRLIIENLMK according to SED ID NO:25, where the spacing and identity of the underlined amino acids are identical or are replaced by conserved substitutions.

20. A method according to claim 19, characterized in that said plant is a member of the Brassicaceae.

21. A method according to claim 19, characterized in that said plant is selected from the group consisting of Arabidopsis thaliana, borage (*Borago* spp.), Canola, castor (*Ricinus communis*), cocoa bean (*Theobroma cacao*), corn (*Zea mays*), cotton (*Gossypium* spp), *Crambe* spp., *Cuphea* spp., flax (*Linum* spp.), *Lesquerella* and *Limnanthes* spp., Linola, nasturtium (*Tropaeolum* spp.), *Oenothera* spp., olive (*Olea* spp.), palm (*Elaeis* spp.), peanut (*Arachis* spp.), rapeseed, safflower (*Carthamus* spp.), soybean (*Glycine* and *Soja* spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.), *Vernonia* spp., wheat (*Triticum* spp.), barley (*Hordeum* spp.), rice (*Oryza* spp.), oat (*Avena* spp.) sorghum (*Sorghum* spp.), rye (*Secale* spp.) and other members of the *Gramineae*.

22. A plant DNA sequence or part thereof, characterized in that the sequence is substantially homologous to at least a part of SEQ ID NO:1 or SEQ ID NO:3, and in that said sequence has been isolated, characterized or designed using sequence information from SEQ ID NO:1 or SEQ ID NO:3, or SEQ ID NO:1 containing an 81 bp insertion [SEQ ID NO:23] such that the deduced amino acid sequence of the encoded protein contains the repeated sequence SHAGLFNLCVVVLIAVNSRLIIENLMK according to SED ID NO:25, where the spacing and identity of the underlined amino acids are identical or are replaced by conserved substitutions.

23. A method of changing the oil content, acyl composition or diacylglycerol/ triacylglycerol proportions of the seed oil of plant seeds by introducing a sense or anti-sense nucleic acid construct into a plant transformation vector, using the vector to transform the genome of a plant or plant seed, and then growing the plant or plant seed and extracting the oil from the plant seed, characterized in that said nucleic acid sequence is SEQ ID NO:1 or SEQ ID NO:3, or a part of SEQ ID NO:1 or SEQ ID NO:3, or a sequence that is substantially homologous to SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:1 [SEQ ID NO:23] containing an 81 bp insertion such that the deduced amino acid sequence of the encoded protein contains the repeated sequence SHAGLFNLCVVVLIAVNSRLIIENLMK according to SED ID NO:25, where the spacing and identity of the underlined amino acids are identical or are replaced by conserved substitutions.

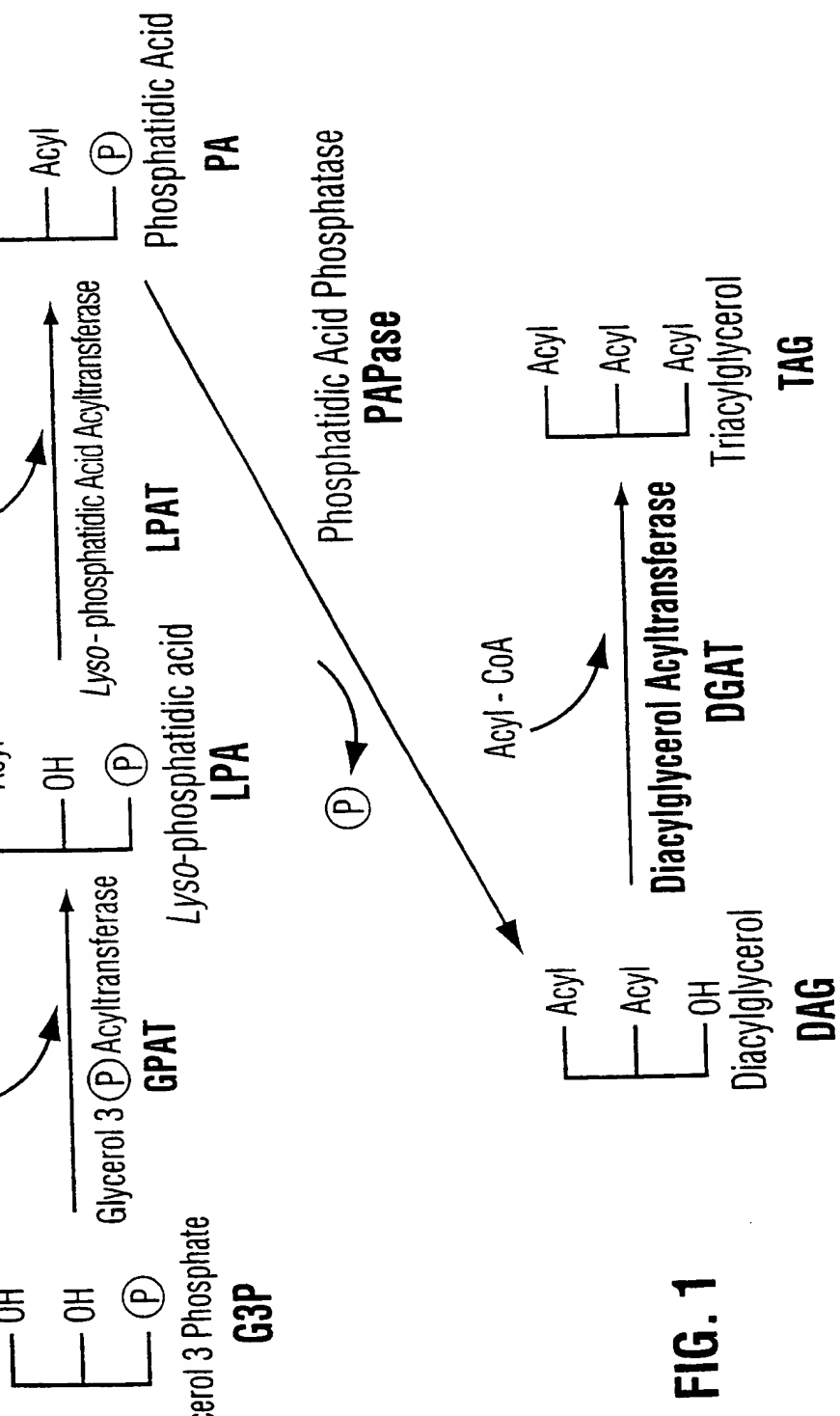

Acyl - CoA
Glycerol 3 P Acyltransferase
GPAT
OH
OH
P
Glycerol 3 Phosphate
G3P
Acyl
OH
P
Lyso-phosphatidic acid
LPA
Acyl - CoA
Lyso-phosphatidic Acid Acyltransferase
LPAT
Acyl
Acyl
P
Phosphatidic Acid
PA
Phosphatidic Acid Phosphatase
PAPase
P
Acyl - CoA
Diacylglycerol Acyltransferase
DGAT
Acyl
Acyl
OH
Diacylglycerol
DAG
Acyl
Acyl
Acyl
Triacylglycerol
TAG

FIG. 1

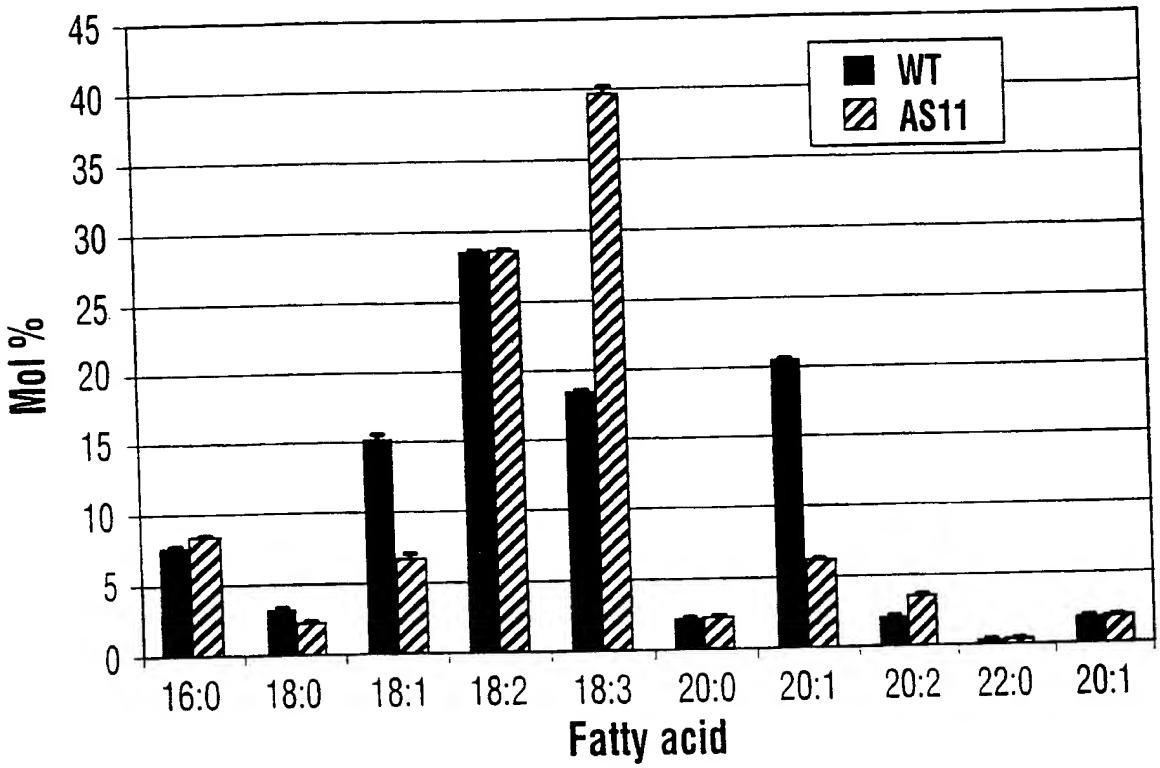

45
40
35
30
25
20
15
10
5
0
Mol %
WT
AS11
16:0
18:0
18:1
18:2
18:3
20:0
20:1
20:2
22:0
20:1
Fatty acid

FIG. 2

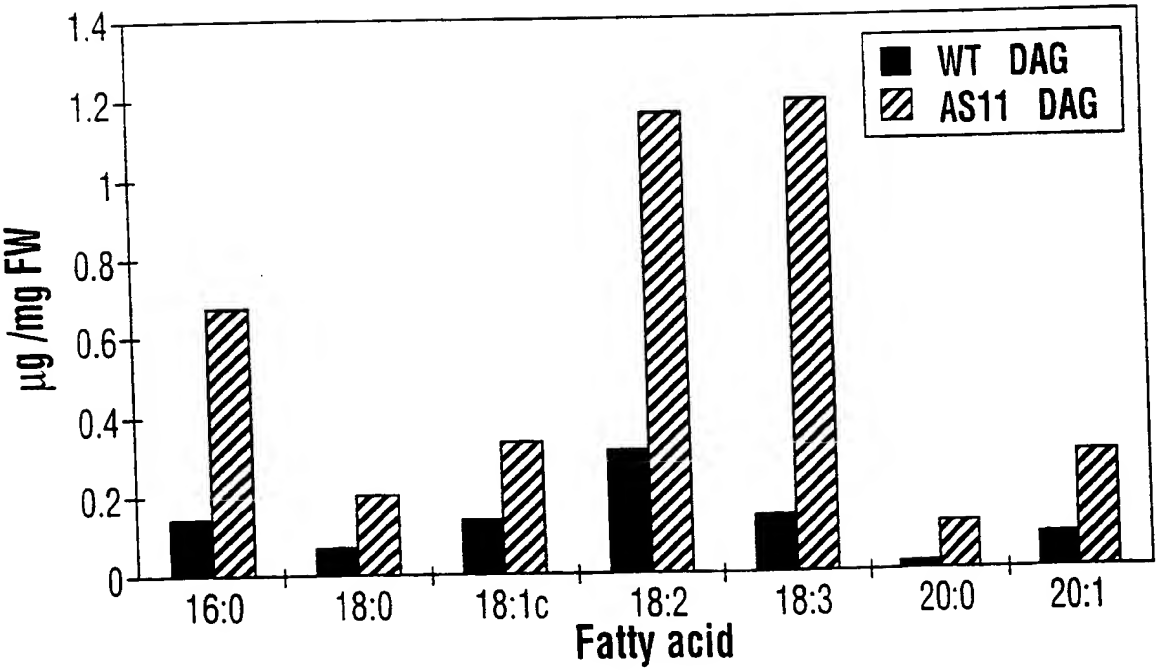

1.4
1.2
1
0.8
0.6
0.4
0.2
0
µg /mg FW
WT DAG
AS11 DAG
16:0
18:0
18:1c
18:2
18:3
20:0
20:1
Fatty acid

FIG. 3

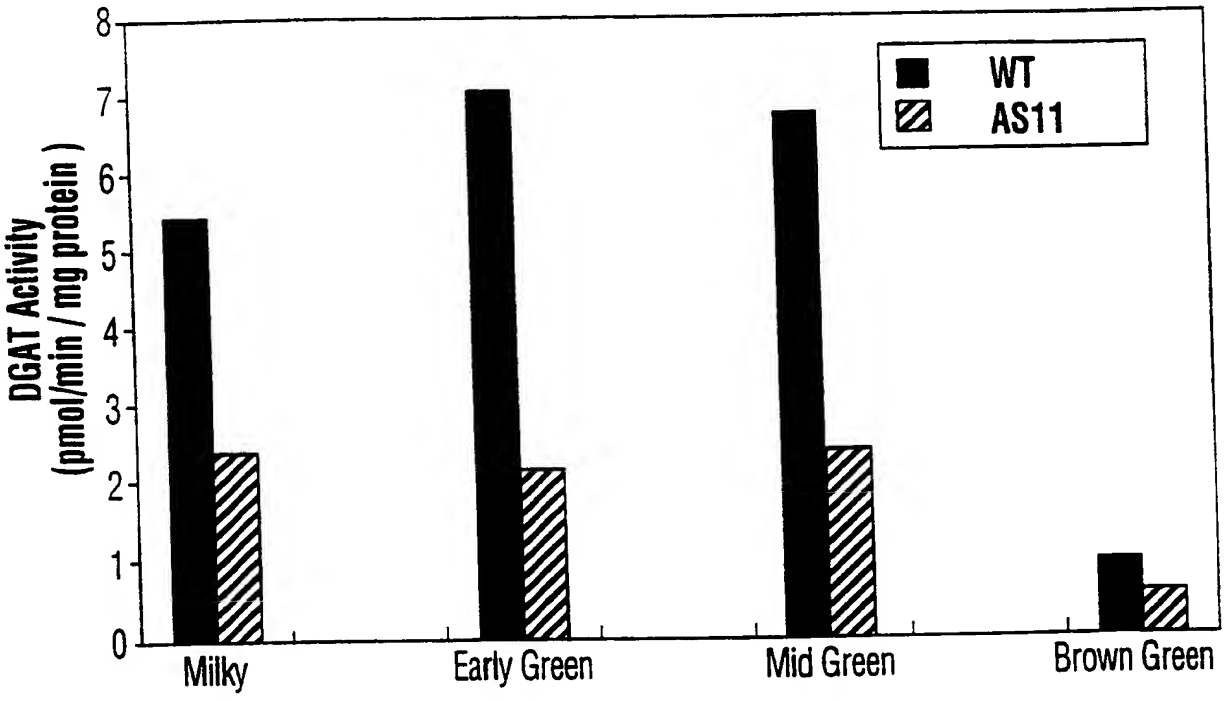

DGAT Activity
(pmol/min / mg protein)
0
1
2
3
4
5
6
7
8
WT
AS11
Milky
Early Green
Mid Green
Brown Green
Developmental Stage

FIG. 4

(a)

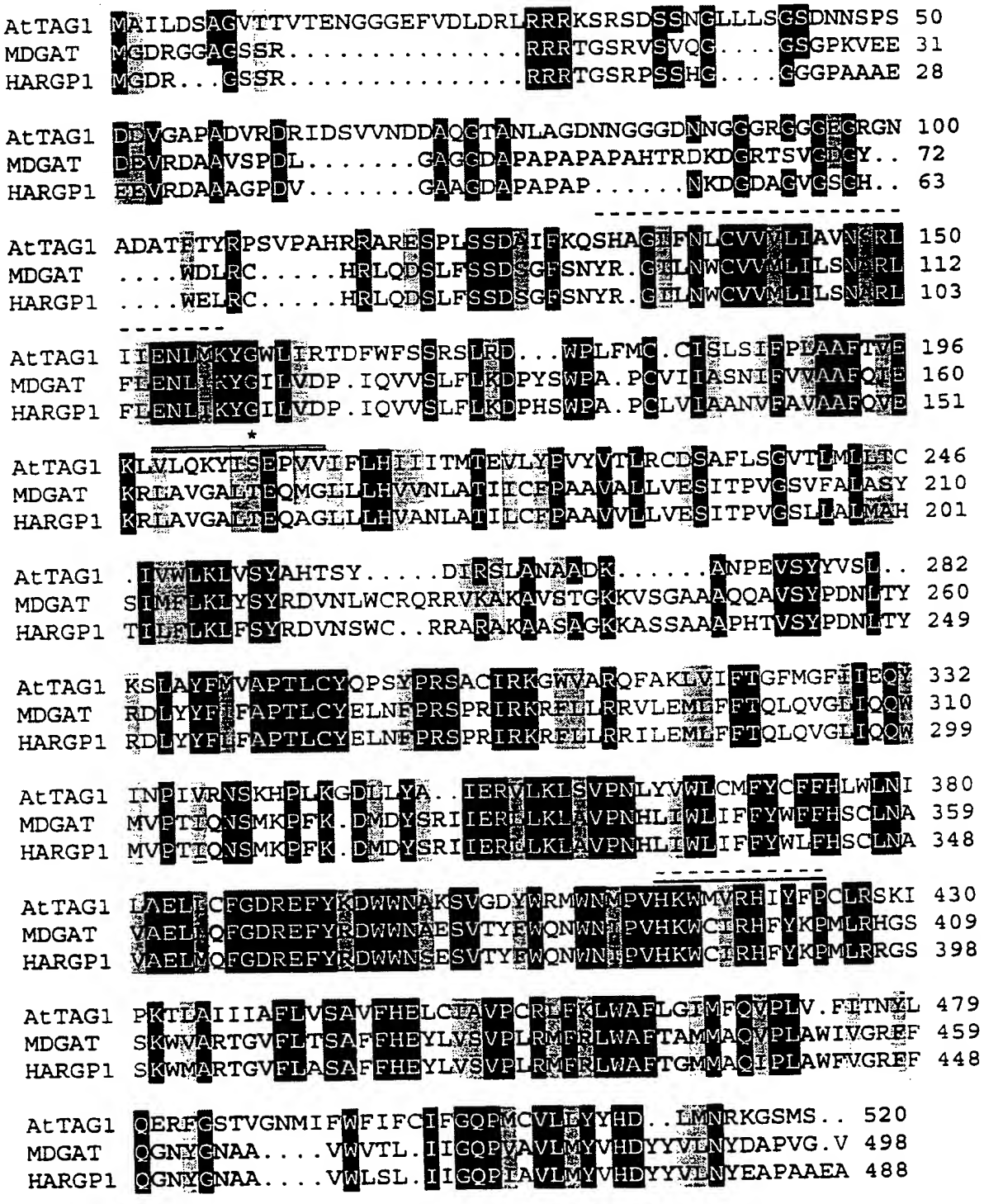

AtTAG1 MAILDSAGVTTVTENGGGEFVDLDRLRRRKSRSDSSNGLLLSGSDNNSPS 50
MDGAT MGDRGGAGSSR.....RRRTGSRVSVQG....GSGPKVEE 31
HARGP1 MGDR...GSSR.....RRRTGSRPSSHG....GGGPAAAE 28
AtTAG1 DDVGAPADVRDRIDSVVNDDAQGTANLAGDNNGGGDNNGGGRGGGEGRGN 100
MDGAT DEVRDAAVSPDL.....GAGGDAPAPAPAPAHTRDKDGRTSVGDGY.. 72
HARGP1 EEVRDAAAGPDV.....GAAGDAPAPAP.....NKDGDAGVGSGH.. 63
AtTAG1 ADATETYRPSVPAHRRARESPLSSDAIFKQSHAGIFNLCVVVLIAVNSRL 150
MDGAT ....WDLRC.....HRLQDSLFSSDSGFSNYR.GILNWCVVMLILSNSRL 112
HARGP1 ....WELRC.....HRLQDSLFSSDSGFSNYR.GILNWCVVMLILSNSRL 103
AtTAG1 IIENLMKYGWLIRTDFWFSSRSLRD...WPLFMC.CISLSIFPLAAFTVE 196
MDGAT FLENLIKYGILVDP.IQVVSLFLKDPYSWPA.PCVIIASNIFVVAAFQIE 160
HARGP1 FLENLIKYGILVDP.IQVVSLFLKDPHSWPA.PCLVIAANVFAVAAFQVE 151
AtTAG1 KLVLQKYISEPVVIFLHIIITMTEVLYPVYVTLRCDSAFLSGVTLMLTIC 246
MDGAT KRLAVGALTEQMGLLLHVVNLATIICFPAAVALLVESITPVGSVFALASY 210
HARGP1 KRLAVGALTEQAGLLLHVANLATIICFPAAVVLLVESITPVGSLLALMAH 201
AtTAG1 .IVWLKLVSYAHTSY.....DIRSLANAADK.....ANPEVSYYVSL.. 282
MDGAT SIMFLKLYSYRDVNLWCRQRRVKAKAVSTGKKVSGAAAQQAVSYPDNLTY 260
HARGP1 TILFLKLFSYRDVNSWC..RRARAKAASAGKKASSAAAPHTVSYPDNLTY 249
AtTAG1 KSLAYFMVAPTLCYQPSYPRSACIRKGWVARQFAKLVIFTGFMGFIIEQY 332
MDGAT RDLYYFLFAPTLCYELNFPRSPRIRKRFLLRRVLEMLFFTQLQVGLIQQW 310
HARGP1 RDLYYFLFAPTLCYELNFPRSPRIRKRFLLRRILEMLFFTQLQVGLIQQW 299
AtTAG1 INPIVRNSKHPLKGDLLYA..IERVLKLSVPNLYVWLCMFYCFFHLWLNI 380
MDGAT MVPTIQNSMKPFK.DMDYSRIIERELKLAVPNHLIWLIFFYWFFHSCLNA 359
HARGP1 MVPTIQNSMKPFK.DMDYSRIIERELKLAVPNHLIWLIFFYWLFHSCLNA 348
AtTAG1 LAELLCFGDREFYKDWWNAKSVGDYWRMWNMPVHKWMVRHIYFPCLRSKI 430
MDGAT VAELLQFGDREFYRDWWNAESVTYEWQNWNIPVHKWCIRHFYKPMLRHGS 409
HARGP1 VAELMQFGDREFYRDWWNSESVTYEWQNWNIPVHKWCIRHFYKPMLRRGS 398
AtTAG1 PKTLAIIIAFLVSAVFHELCIAVPCRLFKLWAFLGIMFQVPLV.FITNYL 479
MDGAT SKWVARTGVFLTSAFFHEYLVSVPLRMFRLWAFTAMMAQVPLAWIVGREF 459
HARGP1 SKWMARTGVFLASAFFHEYLVSVPLRMFRLWAFTGMMAQIPLAWFVGREF 448
AtTAG1 QERFGSTVGNMIFWFIFCIFGQPMCVLLYYHD..LMNRKGSMS.. 520
MDGAT QGNYGNAA....VWVTL.IIGQPVAVLMYVHDYYVLNYDAPVG.V 498
HARGP1 QGNYGNAA....VWLSL.IIGQPIAVLMYVHDYYVLNYEAPAAEA 488

FIG. 5(a)

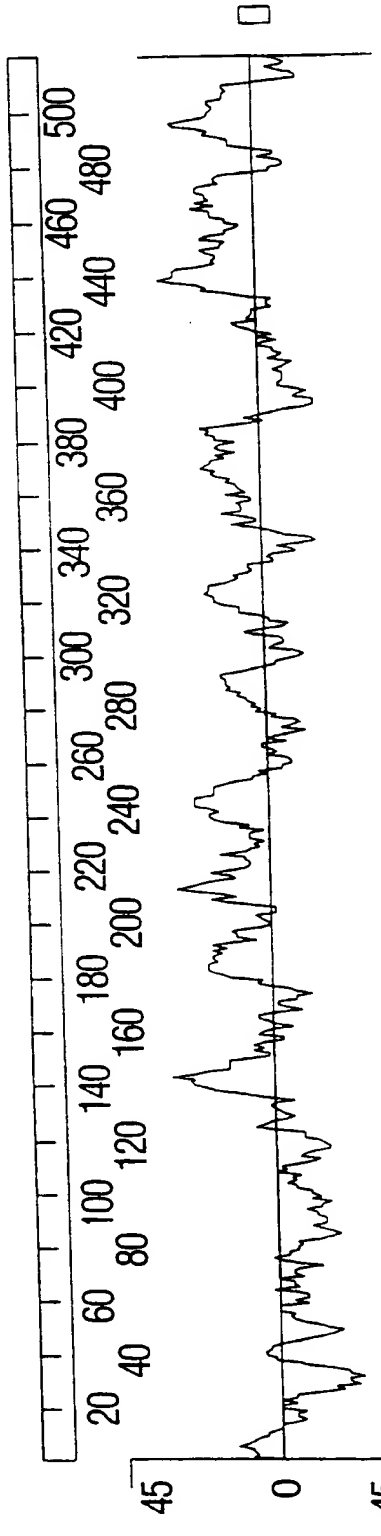

20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
45
0
-45

FIG. 5(b)

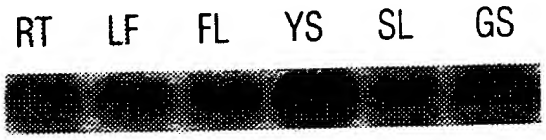


FIG. 6(a)

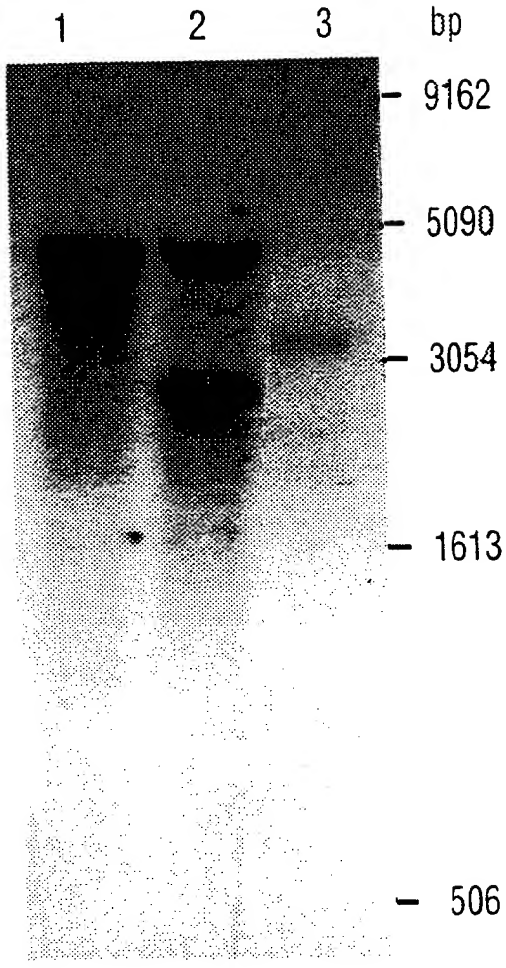


FIG. 6(b)

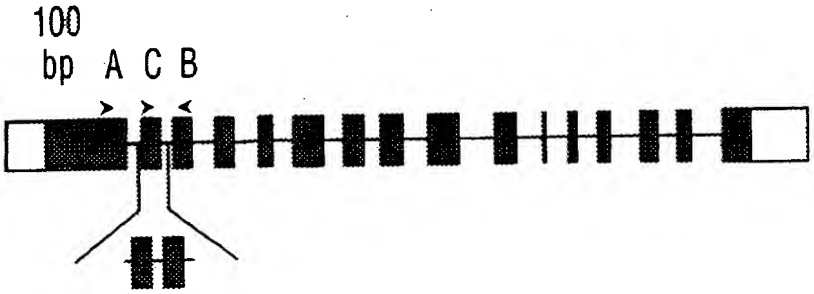


FIG. 7(a)

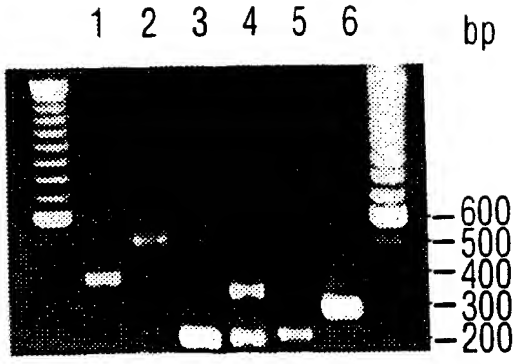


FIG. 7(b)

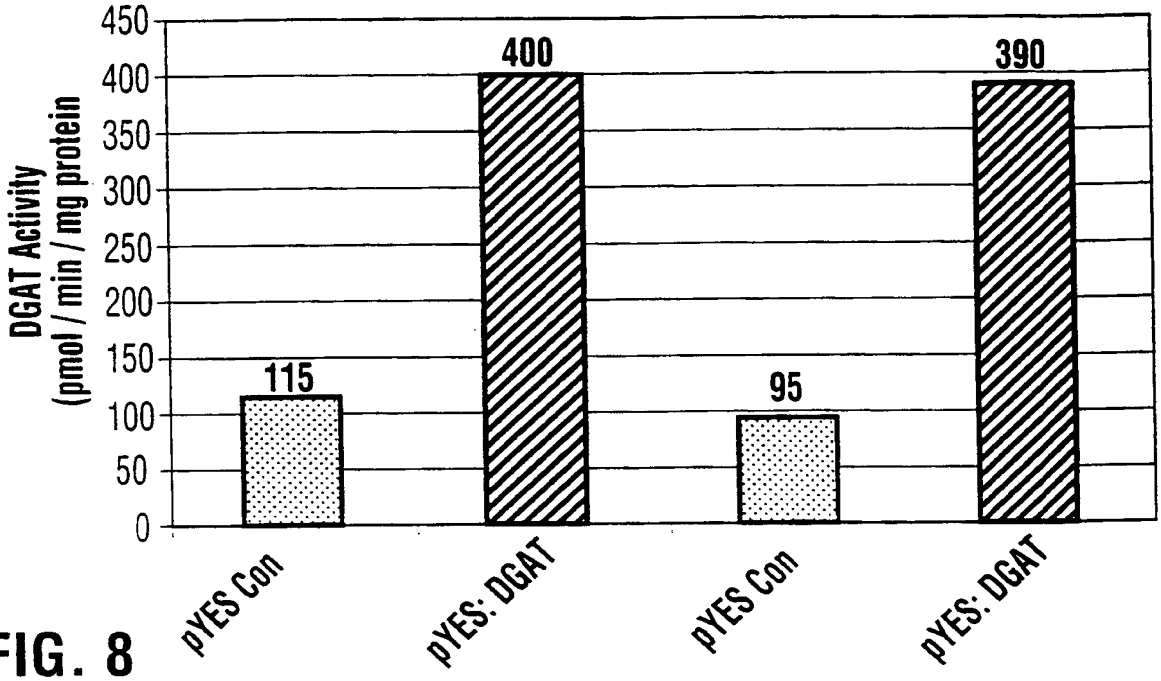

DGAT Activity
(pmol / min / mg protein
450
400
350
300
250
200
150
100
50
0
115
400
95
390
pYES Con
pYES: DGAT
pYES Con
pYES: DGAT

FIG. 8

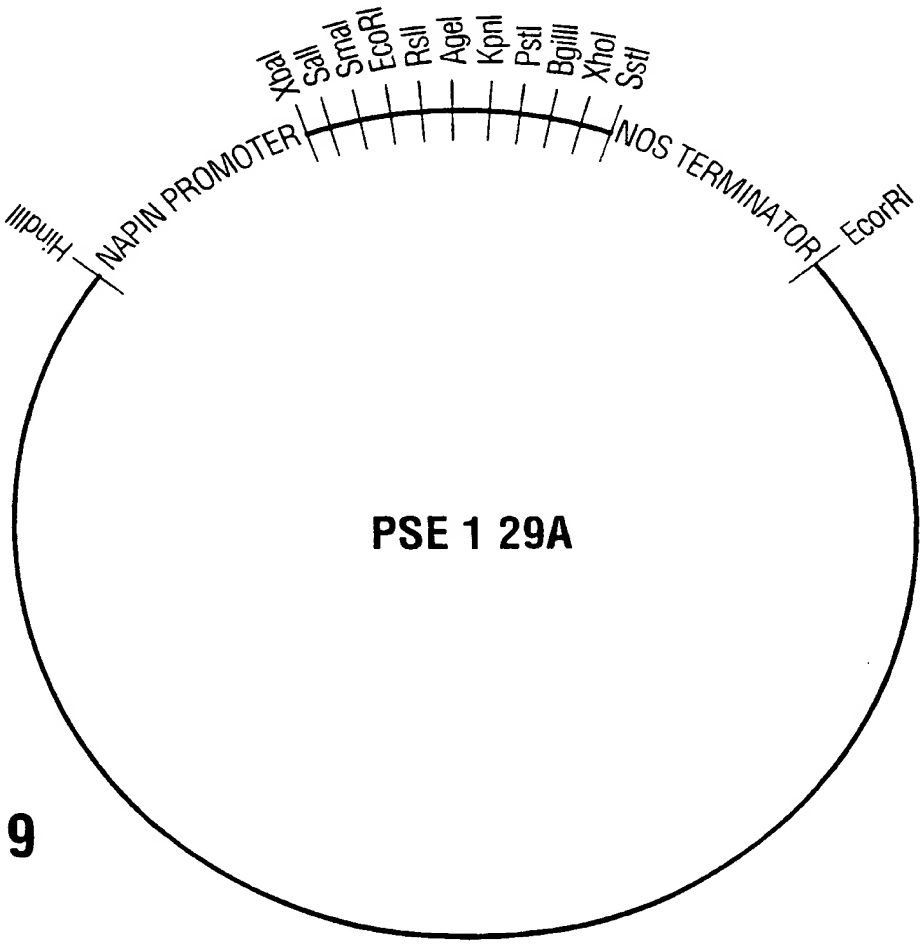

XbaI
SalI
SmaI
EcoRI
RsII
AgeI
KpnI
PstI
BglIII
XhoI
SstI
NAPIN PROMOTER
NOS TERMINATOR
HindIII
EcorRI
PSE 1 29A

FIG. 9

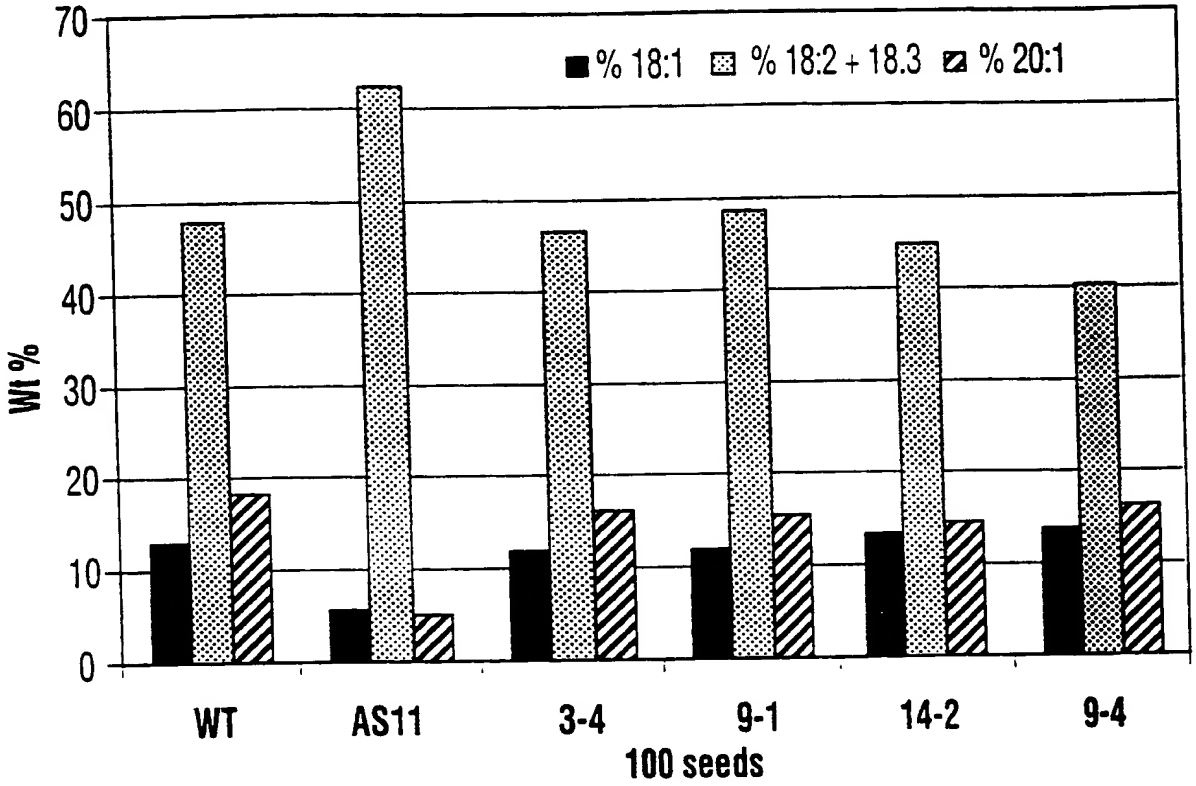

70
60
50
40
30
20
10
0
Wt %
% 18:1
% 18:2 + 18.3
% 20:1
WT
AS11
3-4
9-1
14-2
9-4
100 seeds

FIG. 10

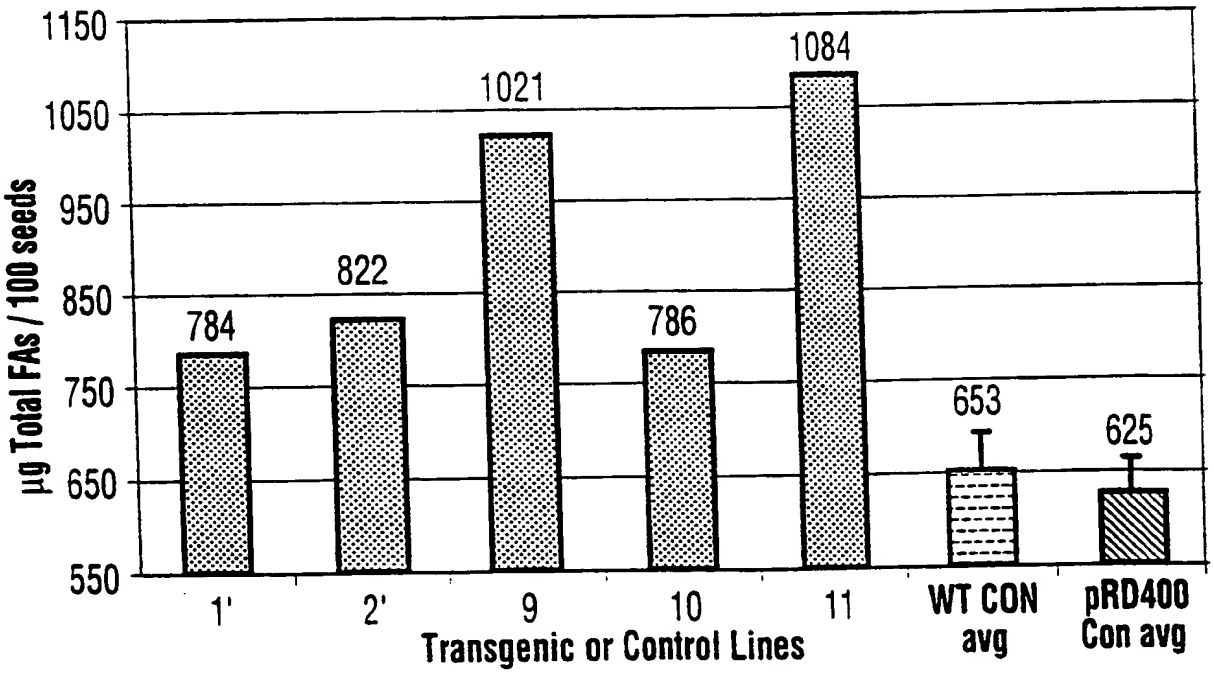

1150
1050
950
850
750
650
550
µg Total FAs / 100 seeds
784
822
1021
786
1084
653
625
1'
2'
9
10
11
WT CON avg
pRD400 Con avg
Transgenic or Control Lines

FIG. 11

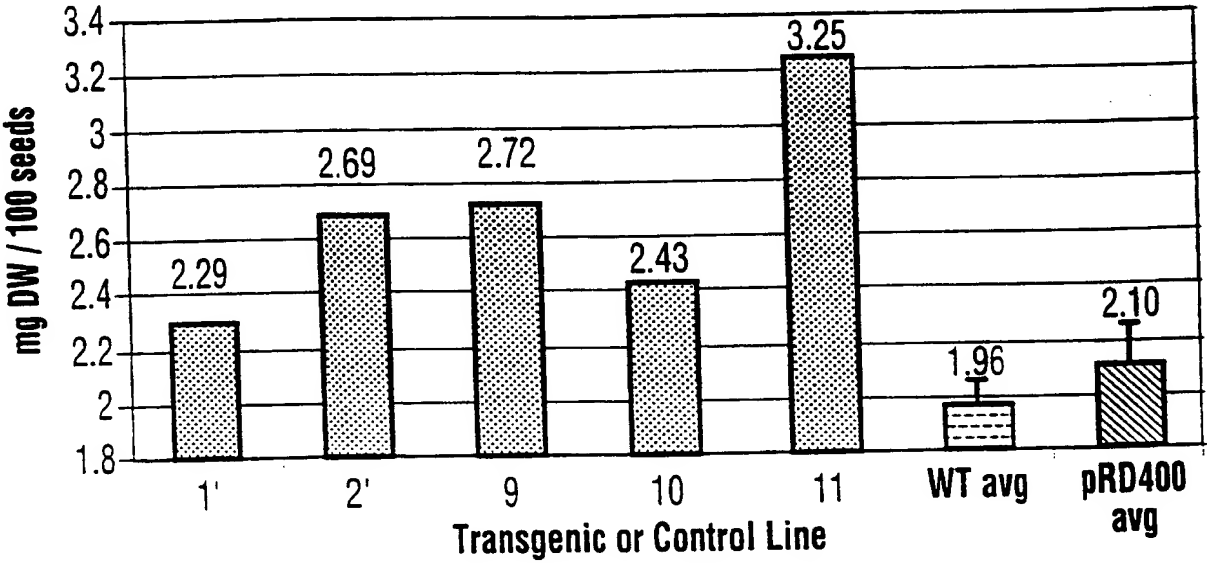

mg DW / 100 seeds
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
2.29
2.69
2.72
2.43
3.25
1.96
2.10
1'
2'
9
10
11
WT avg
pRD400 avg
Transgenic or Control Line

FIG. 12

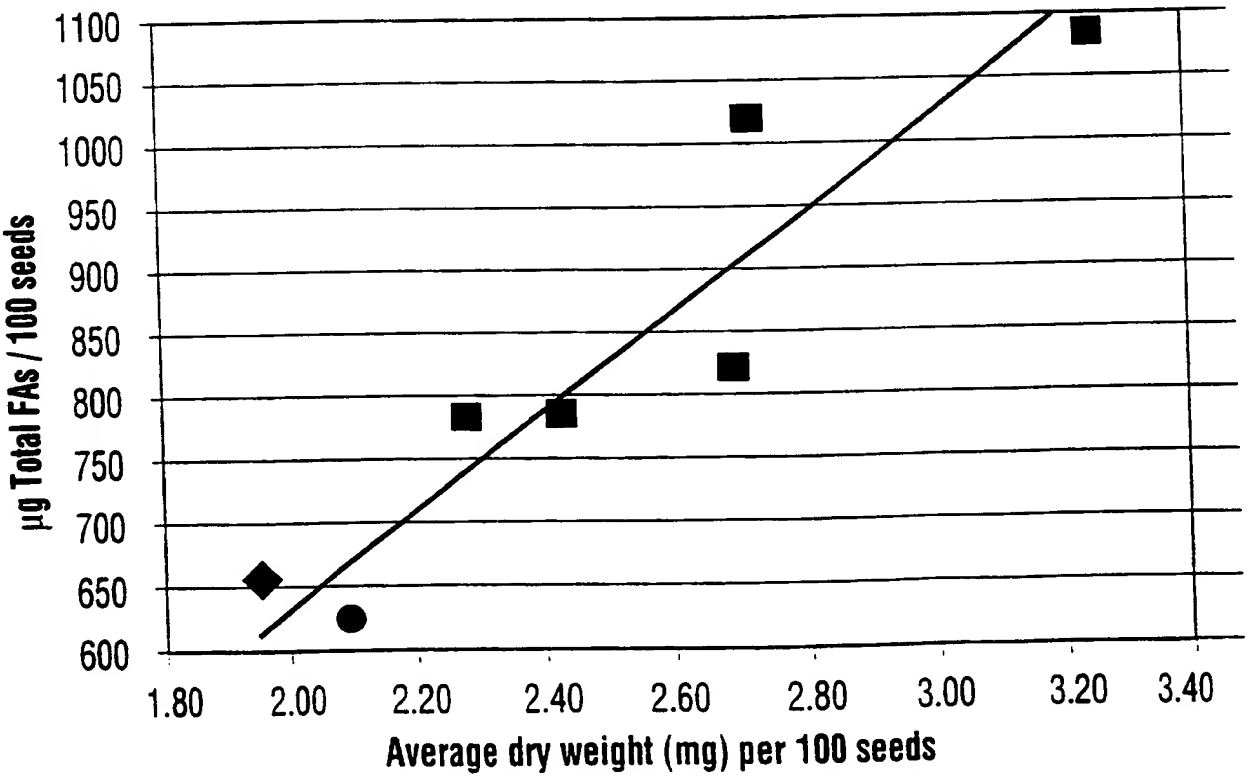

µg Total FAs / 100 seeds
1100
1050
1000
950
900
850
800
750
700
650
600
1.80
2.00
2.20
2.40
2.60
2.80
3.00
3.20
3.40
Average dry weight (mg) per 100 seeds

FIG. 13

SEQUENCE LISTING

<110> National Research Council of Canada
Zou, Jitao
Taylor, David C
Wei, Yangdou
Jako, Colette C

<120> Diacylglycerol Acyl Transferase Gene from Plants

<130> 43922pt

<140> PCT/CA
<141> 1999-12-16

<150> 60/112,812
<151> 1998-12-17

<160> 25

<170> PatentIn Ver. 2.1

<210> 1
<211> 1904
<212> DNA
<213> Arabidopsis thaliana

<400> 1
atttcttagc ttcttccttc aatccgctct ttccctctcc attagattct gtttcctctt 60
tcaatttctt ctgcatgctt ctcgattctc tctgacgcct cttttctccc gacgctgttt 120
cgtcaaacgc ttttcgaaat ggcgattttg gattctgctg gcgttactac ggtgacggag 180
aacggtggcg gagagttcgt cgatcttgat aggcttcgtc gacggaaatc gagatcggat 240
tcttctaacg gacttcttct ctctggttcc gataataatt ctccttcgga tgatgttgga 300
gctcccgccg acgttaggga tcggattgat tccgttgtta acgatgacgc tcagggaaca 360
gccaatttgg ccggagataa taacggtggt ggcgataata acggtggtgg aagaggcggc 420
ggagaaggaa gaggaaacgc cgatgctacg tttacgtatc gaccgtcggt tccagctcat 480
cggagggcga gagagagtcc acttagctcc gacgcaatct tcaaacagag ccatgccgga 540
ttattcaacc tctgtgtagt agttcttatt gctgtaaaca gtagactcat catcgaaaat 600
cttatgaagt atggttggtt gatcagaacg gatttctggt ttagttcaag atcgctgcga 660
gattggccgc ttttcatgtg ttgtatatcc ctttcgatct ttcctttggc tgcctttacg 720
gttgagaaat tggtacttca gaaatacata tcagaacctg ttgtcatctt tcttcatatt 780
attatcacca tgacagaggt tttgtatcca gtttacgtca ccctaaggtg tgattctgct 840
tttttatcag gtgtcacttt gatgctcctc acttgcattg tgtggctaaa gttggtttct 900
tatgctcata ctagctatga cataagatcc ctagccaatg cagctgataa ggccaatcct 960
gaagtctcct actacgttag cttgaagagc ttggcatatt tcatggtcgc tcccacattg 1020
tgttatcagc caagttatcc acgttctgca tgtatacgga agggttgggt ggctcgtcaa 1080
tttgcaaaac tggtcatatt caccggattc atgggattta taatagaaca atatataaat 1140
cctattgtca ggaactcaaa gcatcctttg aaaggcgatc ttctatatgc tattgaaaga 1200
gtgttgaagc tttcagttcc aaatttatat gtgtggctct gcatgttcta ctgcttcttc 1260
caccttttggt taaacatatt ggcagagctt ctctgcttcg gggatcgtga attctacaaa 1320
gattggtgga atgcaaaaag tgtgggagat tactggagaa tgtggaatat gcctgttcat 1380
aaatggatgg ttcgacatat atacttcccg tgcttgcgca gcaagatacc aaagacactc 1440
gccattatca ttgctttcct agtctctgca gtctttcatg agctatgcat cgcagttcct 1500
tgtcgtctct tcaagctatg ggcttttctt gggattatgt ttcaggtgcc tttggtcttc 1560
atcacaaact atctacagga aaggtttggc tcaacggtgg ggaacatgat cttctggttc 1620
atcttctgca ttttcggaca accgatgtgt gtgcttcttt attaccacga cctgatgaac 1680

cgaaaaggat cgatgtcatg aaacaactgt tcaaaaaatg actttcttca aacatctatg 1740
gcctcgttgg atcttccgttg atgttgtggt ggttctgatg ctaaaacgac aaatagtgtt 1800
ataaccattg aagaagaaaa gaaaattaga gttgttgtat ctgcaaaaat tttggtagag 1860
acacgcaaac ccgtttggat tttgttatgg tgtaaagcgg ccgc 1904

<210> 2
<211> 520
<212> PRT
<213> Arabidopsis thaliana

<400> 2

Met Ala Ile Leu Asp Ser Ala Gly Val Thr Thr Val Thr Glu Asn Gly
1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg
20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser
35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp
50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp
65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu
85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro
100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe
115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile
130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp
145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp
165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala
180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val
195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro
210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr
225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala
245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala
260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe
275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala
290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile
305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile
325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile
340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys
355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu
370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys
385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp
405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys
420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu
435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu
450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln
465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe
485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu
500 505 510

Met Asn Arg Lys Gly Ser Met Ser
515 520

<210> 3
<211> 5193
<212> DNA
<213> Arabidopsis thaliana

<400> 3
gctcacgacc cattcttccc gttccatttg gttttattta tttcaaagtt taatattcct 60
tttgtataac attcaaatct tcacatgatt gattgtgtga aaaccccaca gattttacta 120
caatagggg agttgactta aaatagctat tgatgtcgaa aaaatgtatt ttagttataa 180
attatactaa agaaaatttt tgatttgtct gttgtttaag catatgtatt gttaaactta 240
aaaaaatatg tattgttaat cttaaaaatg taggagtaca catcaaatac tcgagcataa 300
tcaaaaccgt attcatagac cgatgtgaga atcaaataga agataatgtg atttttaaa 360
atatcgtatc tccaaatcaa tcacttagaa gataatgtaa ttctttatgt gctacataaa 420
taaatatata tatatatata tatatatatc ttgtatatat gtcttgacaa aaaattgcca 480
gtcaaaaacc atgactgaat caaactataa gtcggattga atcaaactat aagtcggatg 540
agtattaatt tccattatgt ttctatactt tacaaaccgg aaaatagata ttatagatac 600
caaaaaagta gatttgtgta tattattaga agatttggaa tttcatcatt atcaggatct 660
aaagtacttc cctaattaaa tcatgtcggt tgaaaaagct caatgaatgt ttgaaatttg 720
gaaagtttat taaattcgga tctttttttt ttgtttgtcg tcccaaacat ttttatttta 780
ttacaaataa tcaacttatc cttactacta aatcatttca tatctttgat accaacaaat 840
catttcatat tctattttga tgtttaagaa aacactattt accagttaca aaatattata 900
aggattgttg tttagaaaaa aaagtacaag ttgaattctt tttgtcaaat ataaaattga 960
ctttttaata tataattgac ttattgaaca tgattacaga attaatcatc tacaaaactt 1020
tccaagttta taataaatac atttcaaaga ctattagttc ttcttaaaat atttctaaaa 1080
gtgatcaaag actaccacat ataattcaga aaaagtagaa gttgatttct ttttgtcaaa 1140
taaataattg acttaaaata gtttggaaag ccattgaact tgattataga attgataatg 1200
tacataaaaa aattccaagt ttataataaa tacatttttc aaatgctata tcagttcttc 1260
ttaaaatatt tcactaaaaa aacactcaaa tatagaataa atttattgaa taacatacca 1320
actgtaaaac agaatttgac aaaaaaaaaa aaaaaatgaa atgaagatga agacaaaaat 1380
aaatcaccag aggatcttat gcaaaaaaat atatgaatac acaataaacc atattgatat 1440
ttttaaaata aaataaaaac agaaaaatat cccaacaccg cttttcaatt aaaaatcttc 1500
cgtcaccatt gttgtcatct tcctctctcg tgaatccttt ttccttttctt cttcttcttc 1560
tcttcagaga aaactttgct tctctttcta taaggaacca gacacgaatc ccattcccac 1620
cgatttctta gcttcttcct tcaatccgct ctttcccctct ccattagatt ctgtttcctc 1680
tttcaatttc ttctgcatgc ttctcgattc tctctgacgc ctctttttctc ccgacgctgt 1740
ttcgtcaaac gcttttcgaa atggcgattt tggattctgc tggcgttact acggtgacgg 1800
agaacggtgg cggagagttc gtcgatcttg ataggcttcg tcgacggaaa tcgagatcgg 1860
attcttctaa cggacttctt ctctctggtt ccgataataa ttctccttcg gatgatgttg 1920
gagctcccgc cgacgttagg gatcggattg attccgttgt taacgatgac gctcagggaa 1980
cagccaattt ggccggagat aataacggtg gtggcgataa taacggtggt ggaagaggcg 2040
gcggagaagg aagaggaaac gccgatgcta cgtttacgta tcgaccgtcg gttccagctc 2100
atcggagggc gagagagagt ccacttagct ccgacgcaat cttcaaacag gtttaaaatc 2160
tcagaaatct tcgaatttgg tgtttgcttg ttgttttata tggaattgag tttggtgatt 2220
gttttgcatt gcagagccat gccggattat tcaacctctg tgtagtagtt cttattgctg 2280
taaacagtag actcatcatc gaaaatctta tgaaggtttg ctgttacttg tttctccttt 2340
taggaattga attgcttgaa aatttatcag agacgaataa ctttgttgtt gctatcattc 2400
atgtagtatg gttggttgat cagaacggat ttctggttta gttcaagatc gctgcgagat 2460
tggccgcttt tcatgtgttg gtaaaagaag atgtttttta tttccagcaa tgttacattg 2520
ttatacgtat aatgatgagt ttagtgatca agttcctctt tgattcttct ttcttgttgc 2580
agtatatccc tttcgatctt tcctttggct gcctttacgg ttgagaaatt ggtacttcag 2640
aaatacatat cagaacctgt gagtaattac tattctccag ccattactgt aatttttatt 2700
gaagacaagt ttgtatcatg aagaacttac aagttctgtt ttgaaaatgc tcaaggttgt 2760
catctttctt catattatta tcaccatgac agaggttttg tatccagttt acgtcaccct 2820
aaggtgatac tgtttttctg gtctcagttt gtgatactgt ttttaagttt agttgtctga 2880
cccggtgatc ttgaaaatgg acaggtgtga ttctgctttt ttatcaggtg tcactttgat 2940
gctcctcact tgcattgtgt ggctaaagtt ggtttcttat gctcatacta gctatgacat 3000

aagatcccta gccaatgcag ctgataaggt aaaatacgaa aaagaagcgt atgtattagt 3060
cacttgcact gtgttactgt tttaaccaaa cactgttatg aactttaggc caatcctgaa 3120
gtctcctact acgttagctt gaagagcttg gcatatttca tggtcgctcc cacattgtgt 3180
tatcaggtaa ctgcaaagtg catcaaccat tcttatactt gcaagagttt cttgtctaaa 3240
cctcggatct ttgcttttcc ccagccaagt tatccacgtt ctgcatgtat acggaagggt 3300
tgggtggctc gtcaatttgc aaaactggtc atattcaccg gattcatggg atttataata 3360
gaacaagtac gttttcacat cttgctttat tagttttcct tggtgaaaat catcatccct 3420
gcgttgtcac cacttgactt catgttcttt tgttacattt tggcagtata taaatcctat 3480
tgtcaggaac tcaaagcatc ctttgaaagg cgatcttcta tatgctattg aaagagtgtt 3540
gaagctttca gttccaaatt tatatgtgtg gctctgcatg ttctactgct tcttccacct 3600
ttggtatgct gtgatcccat ctctttcaaa ataatttgca aattcgaaaa accgaaaaag 3660
gctaaatctc atacgaattt gatatttta gtttcttaga gtcggtgatg taatttcagt 3720
tactgaacgc aaatctcttg tccaaaggtt aaacatattg gcagagcttc tctgcttcgg 3780
ggatcgtgaa ttctacaaag attggtggaa tgcaaaaagt gtgggagatg tgagctattt 3840
tactcaaaag aaaacttatg atttttaatg ttgtcgttgt ttttgggtca tctaactaac 3900
caaattcatg tattcactgt cttcctttat cagtactgga gaatgtggaa tatggtatgg 3960
ttctcttcct aaacatcacc ttctttttgta cacaaaatag aagaagagag ctaattaaga 4020
tcttgttttc cttgacagcc tgttcataaa tggatggttc gacatatata cttcccgtgc 4080
ttgcgcagca agataccaaa ggtgagtgag atatataccg atatgcaatt gtcgagattt 4140
gtttctgtga tataaattta accctccaca cacttgtttt tcagacactc gccattatca 4200
ttgctttcct agtctctgca gtctttcatg aggtatacat actttctaca ttgccctgtc 4260
tctagacgca tgaacacacg ctagtgaaag aaatgctaat attcaaagca ttgtttttac 4320
ttaacgatct tgtgttacaa atttcctttt gacagctatg catcgcagtt ccttgtcgtc 4380
tcttcaagct atgggctttt cttgggatta tgtttcaggt taaaaaatta ctaaactgct 4440
gcagtcgatt tttactaaac tctaatctca tattctgacc aaccaatttg tttgagtagg 4500
tgcctttggt cttcatcaca aactatctac aggaaaggtt tggctcaacg gtatgctctc 4560
aaaacccgag aaaatagaac gaataactct ttctttcata gcctagccat ttaaatcgca 4620
atgctgaaac ttaataataa aggtgatctg ttttggaatg ggatcatatt attaggtggg 4680
gaacatgatc ttctggttca tcttctgcat tttcggacaa ccgatgtgtg tgcttcttta 4740
ttaccacgac ctgatgaacc gaaaaggatc gatgtcatga aacaactgtt caaaaaatga 4800
ctttcttcaa acatctatgg cctcgttgga tctccgttga tgttgtggtg gttctgatgc 4860
taaaacgaca aatagtgtta taaccattga agaagaaaag aaaattagag ttgttgtatc 4920
tgcaaaaatt ttggtagaga cacgcgaacc cgtttggatt ttgttatggt gtaaagaaat 4980
ttcaatcaaa aaactgttgt aataattgtt accaaaaaga aatgcttttc tggaaacgag 5040
gggaaaaata gtagttttgt taggttttac tgtttggacc aaatctagta aaaaactttt 5100
tgtaataagg aaaaaaaaag aacaaatgtg ataaatgcat ggggattgta tgaaaccttc 5160
caataaagtt gattggtggt cccgttttgg gga 5193

<210> 4
<211> 498
<212> PRT
<213> mouse

<400> 4
Met Gly Asp Arg Gly Gly Ala Gly Ser Ser Arg Arg Arg Arg Thr Gly
1 5 10 15

Ser Arg Val Ser Val Gln Gly Gly Ser Gly Pro Lys Val Glu Glu Asp
20 25 30

Glu Val Arg Asp Ala Ala Val Ser Pro Asp Leu Gly Ala Gly Gly Asp
35 40 45

Ala Pro Ala Pro Ala Pro Ala Pro Ala His Thr Arg Asp Lys Asp Gly
50 55 60

```
Arg Thr Ser Val Gly Asp Gly Tyr Trp Asp Leu Arg Cys His Arg Leu
 65                  70                  75                  80

Gln Asp Ser Leu Phe Ser Ser Asp Ser Gly Phe Ser Asn Tyr Arg Gly
                85                  90                  95

Ile Leu Asn Trp Cys Val Val Met Leu Ile Leu Ser Asn Ala Arg Leu
            100                 105                 110

Phe Leu Glu Asn Leu Ile Lys Tyr Gly Ile Leu Val Asp Pro Ile Gln
        115                 120                 125

Val Val Ser Leu Phe Leu Lys Asp Pro Tyr Ser Trp Pro Ala Pro Cys
    130                 135                 140

Val Ile Ile Ala Ser Asn Ile Phe Val Val Ala Ala Phe Gln Ile Glu
145                 150                 155                 160

Lys Arg Leu Ala Val Gly Ala Leu Thr Glu Gln Met Gly Leu Leu Leu
                165                 170                 175

His Val Val Asn Leu Ala Thr Ile Ile Cys Phe Pro Ala Ala Val Ala
            180                 185                 190

Leu Leu Val Glu Ser Ile Thr Pro Val Gly Ser Val Phe Ala Leu Ala
        195                 200                 205

Ser Tyr Ser Ile Met Phe Leu Lys Leu Tyr Ser Tyr Arg Asp Val Asn
    210                 215                 220

Leu Trp Cys Arg Gln Arg Arg Val Lys Ala Lys Ala Val Ser Thr Gly
225                 230                 235                 240

Lys Lys Val Ser Gly Ala Ala Ala Gln Gln Ala Val Ser Tyr Pro Asp
                245                 250                 255

Asn Leu Thr Tyr Arg Asp Leu Tyr Tyr Phe Ile Phe Ala Pro Thr Leu
            260                 265                 270

Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro Arg Ile Arg Lys Arg Phe
        275                 280                 285

Leu Leu Arg Arg Val Leu Glu Met Leu Phe Phe Thr Gln Leu Gln Val
    290                 295                 300

Gly Leu Ile Gln Gln Trp Met Val Pro Thr Ile Gln Asn Ser Met Lys
305                 310                 315                 320

Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile Ile Glu Arg Leu Leu Lys
                325                 330                 335

Leu Ala Val Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Phe
            340                 345                 350

Phe His Ser Cys Leu Asn Ala Val Ala Glu Leu Leu Gln Phe Gly Asp
        355                 360                 365
```

Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ala Glu Ser Val Thr Tyr Phe
370 375 380

Trp Gln Asn Trp Asn Ile Pro Val His Lys Trp Cys Ile Arg His Phe
385 390 395 400

Tyr Lys Pro Met Leu Arg His Gly Ser Ser Lys Trp Val Ala Arg Thr
405 410 415

Gly Val Phe Leu Thr Ser Ala Phe Phe His Glu Tyr Leu Val Ser Val
420 425 430

Pro Leu Arg Met Phe Arg Leu Trp Ala Phe Thr Ala Met Met Ala Gln
435 440 445

Val Pro Leu Ala Trp Ile Val Gly Arg Phe Phe Gln Gly Asn Tyr Gly
450 455 460

Asn Ala Ala Val Trp Val Thr Leu Ile Ile Gly Gln Pro Val Ala Val
465 470 475 480

Leu Met Tyr Val His Asp Tyr Tyr Val Leu Asn Tyr Asp Ala Pro Val
485 490 495

Gly Val

<210> 5
<211> 488
<212> PRT
<213> human

<400> 5
Met Gly Asp Arg Gly Ser Ser Arg Arg Arg Arg Thr Gly Ser Arg Pro
1 5 10 15

Ser Ser His Gly Gly Gly Gly Pro Ala Ala Ala Glu Glu Glu Val Arg
20 25 30

Asp Ala Ala Ala Gly Pro Asp Val Gly Ala Ala Gly Asp Ala Pro Ala
35 40 45

Pro Ala Pro Asn Lys Asp Gly Asp Ala Gly Val Gly Ser Gly His Trp
50 55 60

Glu Leu Arg Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp Ser
65 70 75 80

Gly Phe Ser Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met Leu
85 90 95

Ile Leu Ser Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr Gly
100 105 110

Ile Leu Val Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro
115 120 125

His Ser Trp Pro Ala Pro Cys Leu Val Ile Ala Ala Asn Val Phe Ala
130 135 140

Val Ala Ala Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu Thr
145 150 155 160

Glu Gln Ala Gly Leu Leu Leu His Val Ala Asn Leu Ala Thr Ile Leu
165 170 175

Cys Phe Pro Ala Ala Val Val Leu Leu Val Glu Ser Ile Thr Pro Val
180 185 190

Gly Ser Leu Leu Ala Leu Met Ala His Thr Ile Leu Phe Leu Lys Leu
195 200 205

Phe Ser Tyr Arg Asp Val Asn Ser Trp Cys Arg Arg Ala Arg Ala Lys
210 215 220

Ala Ala Ser Ala Gly Lys Lys Ala Ser Ser Ala Ala Ala Pro His Thr
225 230 235 240

Val Ser Tyr Pro Asp Asn Leu Thr Tyr Arg Asp Leu Tyr Tyr Phe Leu
245 250 255

Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro Arg
260 265 270

Ile Arg Lys Arg Phe Leu Leu Arg Arg Ile Leu Glu Met Leu Phe Phe
275 280 285

Thr Gln Leu Gln Val Gly Leu Ile Gln Gln Trp Met Val Pro Thr Ile
290 295 300

Gln Asn Ser Met Lys Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile Ile
305 310 315 320

Glu Arg Leu Leu Lys Leu Ala Val Pro Asn His Leu Ile Trp Leu Ile
325 330 335

Phe Phe Tyr Trp Leu Phe His Ser Cys Leu Asn Ala Val Ala Glu Leu
340 345 350

Met Gln Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser Glu
355 360 365

Ser Val Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys Trp
370 375 380

Cys Ile Arg His Phe Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser Lys
385 390 395 400

Trp Met Ala Arg Thr Gly Val Phe Leu Ala Ser Ala Phe Phe His Glu
405 410 415

```
Tyr Leu Val Ser Val Pro Leu Arg Met Phe Arg Leu Trp Ala Phe Thr
            420                 425                 430

Gly Met Met Ala Gln Ile Pro Leu Ala Trp Phe Val Gly Arg Phe Phe
        435                 440                 445

Gln Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser Leu Ile Ile Gly
    450                 455                 460

Gln Pro Ile Ala Val Leu Met Tyr Val His Asp Tyr Tyr Val Leu Asn
465                 470                 475                 480

Tyr Glu Ala Pro Ala Ala Glu Ala
                485
```

<210> 6
<211> 629
<212> DNA
<213> Arabidopsis thaliana

<400> 6
tgcatgtata cggaagggtt gggtggctcg tcaatttgca aaactggtca tattcaccgg 60
attcatggga tttataatag aacaatatat aaatcctatt gtcaggaact caaagcatcc 120
tttgaaaggc gatcttctat atgctattga aagagtgttg aagctttcag ttccaaattt 180
atatgtgtgg ctctgcatgt tctactgctt cttccacctt tggttaaaca tattggcaga 240
gcttctctgc ttcggggatc gtgaattcta caaagattgg tggaatgcaa aaagtgtggg 300
agattactgg gagaatgtgg aatatgcctg tccataaatg ggatgggtcc gacatatata 360
ccttccccgt gcttgcgcac aaggattacc caaagacacc ccggccatta accattggct 420
ttcccaagcc ccctggaggc ctttccatgg gccanggacc cggngtnccc tggcnggccc 480
ttcaaagcaa agggggnttn cctggggnta aagntccang ggcccttggg gcccanccaa 540
aannttcccc cgggaaaggg ttgcccaccg gggggngaaa aannccgggg ggcaccncgg 600
aattttggga acccgggggg ggccttttt 629

<210> 7
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<400> 7
His Lys Trp Met Val Arg His Ile Tyr Phe Pro
  1   5   10

<210> 8
<211> 341
<212> PRT
<213> Brassica napus

<400> 8
Met Cys Cys Leu Ser Leu Ser Ile Phe Pro Leu Ala Ala Phe Thr Val
  1   5   10   15

Glu Lys Leu Val Leu Gln Lys Cys Ile Ser Glu Pro Val Val Ile Phe
20 25 30

Leu His Val Ile Ile Thr Met Thr Glu Val Leu Tyr Pro Val Tyr Val
35 40 45

Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Asp Thr Leu Met Leu
50 55 60

Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala His Thr Asn
65 70 75 80

Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ser Asp Lys Ala Asn Pro Glu
85 90 95

Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe Met Leu Ala
100 105 110

Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro Cys Ile Arg
115 120 125

Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile Phe Thr Gly
130 135 140

Leu Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile Val Arg Asn
145 150 155 160

Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile Glu Arg Val
165 170 175

Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys Met Phe Tyr
180 185 190

Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu Leu Cys Phe
195 200 205

Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys Ser Val Gly
210 215 220

Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met Val Arg
225 230 235 240

His Val Tyr Phe Pro Cys Leu Arg Ile Lys Ile Pro Lys Val Pro Ala
245 250 255

Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu Leu Cys Ile
260 265 270

Ala Val Pro Cys Arg Leu Phe Asn Leu Trp Ala Phe Met Gly Ile Met
275 280 285

Phe Gln Val Pro Leu Val Phe Ile Thr Asn Phe Leu Gln Glu Arg Phe
290 295 300

Gly Ser Met Val Gly Asn Met Ile Phe Gly Ser Ala Ser Cys Ile Phe
305 310 315 320

Gly Gln Pro Met Cys Gly Leu Leu Tyr Tyr His Asp Leu Met Asn Arg
325 330 335

Lys Gly Ser Met Ser
340

<210> 9
<211> 503
<212> PRT
<213> Brassica napus

<400> 9
Met Ala Ile Leu Asp Ser Gly Gly Val Ala Val Pro Pro Thr Glu Asn
1 5 10 15

Gly Val Ala Asp Leu Asp Arg Leu His Arg Arg Lys Ser Ser Ser Asp
20 25 30

Ser Ser Asn Gly Leu Leu Ser Asp Thr Ser Pro Ser Asp Asp Val Gly
35 40 45

Ala Ala Ala Ala Glu Arg Asp Arg Val Asp Ser Ala Ala Glu Glu Glu
50 55 60

Ala Gln Gly Thr Ala Asn Leu Ala Gly Gly Asp Ala Glu Thr Arg Glu
65 70 75 80

Ser Ala Gly Gly Asp Val Arg Phe Thr Tyr Arg Pro Ser Val Pro Ala
85 90 95

His Arg Arg Thr Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe Lys
100 105 110

Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Val Ala
115 120 125

Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp Leu
130 135 140

Ile Arg Thr Asp Phe Trp Phe Ser Ser Thr Ser Leu Arg Asp Trp Pro
145 150 155 160

Leu Phe Met Cys Cys Leu Ser Leu Ser Val Phe Pro Leu Ala Ala Phe
165 170 175

Thr Val Glu Lys Met Val Leu Gln Lys Phe Ile Ser Glu Pro Val Ala
180 185 190

Ile Ile Leu His Val Ile Ile Thr Met Thr Glu Val Leu Tyr Pro Val
195 200 205

Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr Leu
210 215 220

Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala His
225 230 235 240

Thr Ser Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ala Asp Lys Val Asp
245 250 255

Pro Glu Ile Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe Met
260 265 270

Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro Cys
275 280 285

Ile Arg Lys Gly Trp Val Ala Arg Gln Leu Ala Lys Leu Val Ile Phe
290 295 300

Thr Gly Leu Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile Val
305 310 315 320

Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile Glu
325 330 335

Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys Met
340 345 350

Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu Leu
355 360 365

Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys Ser
370 375 380

Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met
385 390 395 400

Val Arg His Val Tyr Phe Pro Cys Leu Arg Ile Lys Ile Pro Lys Val
405 410 415

Pro Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu Leu
420 425 430

Cys Ile Ala Val Pro Cys Arg Leu Phe Asn Leu Trp Ala Phe Met Gly
435 440 445

Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Phe Leu Gln Glu
450 455 460

Arg Phe Gly Ser Met Val Gly Asn Met Ile Phe Gly Ser Ala Ser Cys
465 470 475 480

Ile Phe Gly Gln Pro Met Cys Gly Leu Leu Tyr Tyr His Asp Leu Met
485 490 495

Asn Arg Lys Gly Ser Met Ser
500

<210> 10
<211> 11
<212> PRT
<213> Arabidopsis thaliana

<220>
<221> VARIANT
<222> (1)

<220>
<221> VARIANT
<222> (3)

<220>
<221> VARIANT
<222> (5)..(6)

<220>
<221> VARIANT
<222> (8)..(10)

<400> 10
Xaa Leu Xaa Lys Xaa Xaa Ser Xaa Xaa Xaa Val
1 5 10

<210> 11
<211> 27
<212> PRT
<213> Arabidopsis thaliana

<400> 11
Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile Ala Val
1 5 10 15

Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys
20 25

<210> 12
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer of DGATXbal

<400> 12
ctagtctaga atggcgattt tgga 24

<210> 13
<211> 23

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer of DGATXhol

<400> 13
gcgctcgagt ttcatgacat cga 23

<210> 14
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer DGAT1

<400> 14
agacacgaat cccattccca ccga 24

<210> 15
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer DGAT2

<400> 15
agtggtgaca acgcagggat gatg 24

<210> 16
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer DGAT3

<400> 16
atggtcgctc ccacattgtg t 21

<210> 17
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer DGAT4

<400> 17

catacaatcc ccatgacatt tatca 25

<210> 18
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer A

<400> 18
cgaccgtcgg ttccagctca tcgg 24

<210> 19
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer B

<400> 19
gcggccaatc tcgcagcgat cttg 24

<210> 20
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer C

<400> 20
taaacagtag actcatcatc g 21

<210> 21
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer Gen 1

<400> 21
gagaggatcc acgctcacga cccattcttc ccg 33

<210> 22
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer Gen 2

<400> 22
aagaaggatc catccccaaa acgggaccac caa 33

<210> 23
<211> 1985
<212> DNA
<213> Arabidopsis thaliana

<400> 23
atttcttagc ttcttccttc aatccgctct ttccctctcc attagattct gtttcctctt 60
tcaatttctt ctgcatgctt ctcgattctc tctgacgcct cttttctccc gacgctgttt 120
cgtcaaacgc ttttcgaaat ggcgattttg gattctgctg gcgttactac ggtgacggag 180
aacggtggcg gagagttcgt cgatcttgat aggcttcgtc gacggaaatc gagatcggat 240
tcttctaacg gacttcttct ctctggttcc gataataatt ctccttcgga tgatgttgga 300
gctcccgccg acgttaggga tcggattgat tccgttgtta acgatgacgc tcagggaaca 360
gccaatttgg ccggagataa taacggtggt ggcgataata acggtggtgg aagaggcggc 420
ggagaaggaa gaggaaacgc cgatgctacg tttacgtatc gaccgtcggt tccagctcat 480
cggagggcga gagagagtcc acttagctcc gacgcaatct tcaaacagag ccatgccgga 540
ttattcaacc tctgtgtagt agttcttatt gctgtaaaca gtagactcat catcgaaaat 600
cttatgaaga gccatgccgg attattcaac ctctgtgtag tagttcttat tgctgtaaac 660
agtagactca tcatcgaaaa tcttatgaag tatggttggt tgatcagaac ggatttctgg 720
tttagttcaa gatcgctgcg agattggccg cttttcatgt gttgtatatc cctttcgatc 780
tttcctttgg ctgcctttac ggttgagaaa ttggtacttc agaaatacat atcagaacct 840
gttgtcatct ttcttcatat tattatcacc atgacagagg ttttgtatcc agtttacgtc 900
accctaaggt gtgattctgc ttttttatca ggtgtcactt tgatgctcct cacttgcatt 960
gtgtggctaa agttggtttc ttatgctcat actagctatg acataagatc cctagccaat 1020
gcagctgata aggccaatcc tgaagtctcc tactacgtta gcttgaagag cttggcatat 1080
ttcatggtcg ctcccacatt gtgttatcag ccaagttatc cacgttctgc atgtatacgg 1140
aagggttggg tggctcgtca atttgcaaaa ctggtcatat tcaccggatt catgggattt 1200
ataatagaac aatatataaa tcctattgtc aggaactcaa agcatccttt gaaaggcgat 1260
cttctatatg ctattgaaag agtgttgaag ctttcagttc caaatttata tgtgtggctc 1320
tgcatgttct actgcttctt ccacctttgg ttaaacatat tggcagagct tctctgcttc 1380
ggggatcgtg aattctacaa agattggtgg aatgcaaaaa gtgtgggaga ttactggaga 1440
atgtggaata tgcctgttca taaatggatg gttcgacata tatacttccc gtgcttgcgc 1500
agcaagatac caaagacact cgccattatc attgctttcc tagtctctgc agtctttcat 1560
gagctatgca tcgcagttcc ttgtcgtctc ttcaagctat gggcttttct tgggattatg 1620
tttcaggtgc ctttggtctt catcacaaac tatctacagg aaaggtttgg ctcaacggtg 1680
gggaacatga tcttctggtt catcttctgc attttcggac aaccgatgtg tgtgcttctt 1740
tattaccacg acctgatgaa ccgaaaagga tcgatgtcat gaaacaactg ttcaaaaaat 1800
gactttcttc aaacatctat ggcctcgttg gatctccgtt gatgttgtgg tggttctgat 1860
gctaaaacga caaatagtgt tataaccatt gaagaagaaa agaaaattag agttgttgta 1920
tctgcaaaaa ttttggtaga gacacgcaaa cccgtttgga ttttgttatg gtgtaaagcg 1980
gccgc 1985

<210> 24
<211> 5339
<212> DNA
<213> Arabidopsis thaliana

<400> 24
gctcacgacc cattcttccc gttccatttg gttttattta tttcaaagtt taatattcct 60

tttgtataac attcaaatct tcacatgatt gattgtgtga aaaccccaca gattttacta 120
caatagggg agttgactta aaatagctat tgatgtcgaa aaaatgtatt ttagttataa 180
attatactaa agaaaatttt tgatttgtct gttgtttaag catatgtatt gttaaactta 240
aaaaaatatg tattgttaat cttaaaaatg taggagtaca catcaaatac tcgagcataa 300
tcaaaaccgt attcatagac cgatgtgaga atcaaataga agataatgtg atttttaaa 360
atatcgtatc tccaaatcaa tcacttagaa gataatgtaa ttctttatgt gctacataaa 420
taaatatata tatatatata tatatatatc ttgtatatat gtcttgacaa aaaattgcca 480
gtcaaaaacc atgactgaat caaactataa gtcggattga atcaaactat aagtcggatg 540
agtattaatt tccattatgt ttctatactt tacaaaccgg aaaatagata ttatagatac 600
caaaaaagta gatttgtgta tattattaga agatttggaa tttcatcatt atcaggatct 660
aaagtacttc cctaattaaa tcatgtcggt tgaaaaagct caatgaatgt ttgaaatttg 720
gaaagtttat taaattcgga tctttttttt ttgtttgtcg tcccaaacat ttttatttta 780
ttacaaataa tcaacttatc cttactacta aatcatttca tatctttgat accaacaaat 840
catttcatat tctattttga tgtttaagaa aacactattt accagttaca aaatattata 900
aggattgttg tttagaaaaa aaagtacaag ttgaattctt tttgtcaaat ataaaattga 960
ctttttaata tataattgac ttattgaaca tgattacaga attaatcatc tacaaaactt 1020
tccaagttta taataaatac atttcaaaga ctattagttc ttcttaaaat atttctaaaa 1080
gtgatcaaag actaccacat ataattcaga aaaagtagaa gttgatttct ttttgtcaaa 1140
taaataattg acttaaaata gtttggaaag ccattgaact tgattataga attgataatg 1200
tacataaaaa aattccaagt ttataataaa tacatttttc aaatgctata tcagttcttc 1260
ttaaaatatt tcactaaaaa aacactcaaa tatagaataa atttattgaa taacatacca 1320
actgtaaaac agaatttgac aaaaaaaaaa aaaaaatgaa atgaagatga agacaaaaat 1380
aaatcaccag aggatcttat gcaaaaaaat atatgaatac acaataaacc atattgatat 1440
ttttaaaata aaataaaaac agaaaaatat cccaacaccg cttttcaatt aaaaatcttc 1500
cgtcaccatt gttgtcatct tcctctctcg tgaatccttt ttcctttctt cttcttcttc 1560
tcttcagaga aaactttgct tctctttcta taaggaacca gacacgaatc ccattcccac 1620
cgatttctta gcttcttcct tcaatccgct ctttccctct ccattagatt ctgtttcctc 1680
tttcaatttc ttctgcatgc ttctcgattc tctctgacgc ctctttttctc ccgacgctgt 1740
ttcgtcaaac gcttttcgaa atggcgattt tggattctgc tggcgttact acggtgacgg 1800
agaacggtgg cggagagttc gtcgatcttg ataggcttcg tcgacggaaa tcgagatcgg 1860
attcttctaa cggacttctt ctctctggtt ccgataataa ttctccttcg gatgatgttg 1920
gagctcccgc cgacgttagg gatcggattg attccgttgt taacgatgac gctcagggaa 1980
cagccaattt ggccggagat aataacggtg gtggcgataa taacggtggt ggaagaggcg 2040
gcggagaagg aagaggaaac gccgatgcta cgtttacgta tcgaccgtcg gttccagctc 2100
atcggagggc gagagagagt ccacttagct ccgacgcaat cttcaaacag gtttaaaatc 2160
tcagaaatct tcgaatttgg tgtttgcttg ttgttttata tggaattgag tttggtgatt 2220
gttttgcatt gcagagccat gccggattat tcaacctctg tgtagtagtt cttattgctg 2280
taaacagtag actcatcatc gaaaatctta tgaaggtttg ctgttacttg tttctccttt 2340
taggaattga attgcttgaa aatttatcat tgcattgcag agccatgccg gattattcaa 2400
cctctgtgta gtagttctta ttgctgtaaa cagtagactc atcatcgaaa atcttatgaa 2460
ggtttgctgt tacttgtttc tccttttagg aattgaattg cttgaaaatt tatcagagac 2520
gaataacttt gttgttgcta tcattcatgt agtatggttg gttgatcaga acggatttct 2580
ggtttagttc aagatcgctg cgagattggc cgcttttcat gtgttggtaa aagaagatgt 2640
tttttatttc cagcaatgtt acattgttat acgtataatg atgagtttag tgatcaagtt 2700
cctctttgat tcttctttct tgttgcagta tatccctttc gatctttcct ttggctgcct 2760
ttacggttga gaaattggta cttcagaaat acatatcaga acctgtgagt aattactatt 2820
ctccagccat tactgtaatt tttattgaag acaagtttgt atcatgaaga acttacaagt 2880
tctgttttga aaatgctcaa ggttgtcatc tttcttcata ttattatcac catgacagag 2940
gttttgtatc cagtttacgt caccctaagg tgatactgtt tttctggtct cagtttgtga 3000
tactgttttt aagtttagtt gtctgacccg gtgatcttga aaatggacag gtgtgattct 3060
gctttttttat caggtgtcac tttgatgctc ctcacttgca ttgtgtggct aaagttggtt 3120
tcttatgctc atactagcta tgacataaga tccctagcca atgcagctga taaggtaaaa 3180
tacgaaaaag aagcgtatgt attagtcact tgcactgtgt tactgtttta accaaacact 3240
gttatgaact ttaggccaat cctgaagtct cctactacgt tagcttgaag agcttggcat 3300
atttcatggt cgctcccaca ttgtgttatc aggtaactgc aaagtgcatc aaccattctt 3360
atacttgcaa gagtttcttg tctaaacctc ggatctttgc ttttccccag ccaagttatc 3420

```
cacgttctgc atgtatacgg aagggttggg tggctcgtca atttgcaaaa ctggtcatat 3480
tcaccggatt catgggattt ataatagaac aagtacgttt tcacatcttg ctttattagt 3540
tttccttggt gaaaatcatc atccctgcgt tgtcaccact tgacttcatg ttcttttgtt 3600
acatttttggc agtatataaa tcctattgtc aggaactcaa agcatccttt gaaaggcgat 3660
cttctatatg ctattgaaag agtgttgaag ctttcagttc caaatttata tgtgtggctc 3720
tgcatgttct actgcttctt ccacctttgg tatgctgtga tcccatctct ttcaaaataa 3780
tttgcaaatt cgaaaaaccg aaaaaggcta aatctcatac gaatttgata tttttagttt 3840
cttagagtcg gtgatgtaat ttcagttact gaacgcaaat ctcttgtcca aaggttaaac 3900
atattggcag agcttctctg cttcggggat cgtgaattct acaaagattg gtggaatgca 3960
aaaagtgtgg gagatgtgag ctattttact caaaagaaaa cttatgattt ttaatgttgt 4020
cgttgttttt gggtcatcta actaaccaaa ttcatgtatt cactgtcttc ctttatcagt 4080
actggagaat gtggaatatg gtatggttct cttcctaaac atcaccttct tttgtacaca 4140
aaatagaaga agagagctaa ttaagatctt gttttccttg acagcctgtt cataaatgga 4200
tggttcgaca tatatacttc ccgtgcttgc gcagcaagat accaaaggtg agtgagatat 4260
ataccgatat gcaattgtcg agatttgttt ctgtgatata aatttaaccc tccacacact 4320
tgtttttcag acactcgcca ttatcattgc tttcctagtc tctgcagtct ttcatgaggt 4380
atacatactt tctacattgc cctgtctcta gacgcatgaa cacacgctag tgaaagaaat 4440
gctaatattc aaagcattgt ttttacttaa cgatcttgtg ttacaaattt ccttttgaca 4500
gctatgcatc gcagttcctt gtcgtctctt caagctatgg gcttttcttg ggattatgtt 4560
tcaggttaaa aaattactaa actgctgcag tcgattttta ctaaactcta atctcatatt 4620
ctgaccaacc aatttgtttg agtaggtgcc tttggtcttc atcacaaact atctacagga 4680
aaggtttggc tcaacggtat gctctcaaaa cccgagaaaa tagaacgaat aactctttct 4740
ttcatagcct agccatttaa atcgcaatgc tgaaacttaa taataaaggt gatctgtttt 4800
ggaatgggat catattatta ggtggggaac atgatcttct ggttcatctt ctgcattttc 4860
ggacaaccga tgtgtgtgct tctttattac cacgacctga tgaaccgaaa aggatcgatg 4920
tcatgaaaca actgttcaaa aaatgacttt cttcaaacat ctatggcctc gttggatctc 4980
cgttgatgtt gtggtggttc tgatgctaaa acgacaaata gtgttataac cattgaagaa 5040
gaaaagaaaa ttagagttgt tgtatctgca aaaattttgg tagagacacg cgaacccgtt 5100
tggattttgt tatggtgtaa agaaatttca atcaaaaaac tgttgtaata attgttacca 5160
aaaagaaatg cttttctgga aacgagggga aaaatagtag ttttgttagg ttttactgtt 5220
tggaccaaat ctagtaaaaa acttttttgta ataaggaaaa aaaaagaaca aatgtgataa 5280
atgcatgggg attgtatgaa accttccaat aaagttgatt ggtggtcccg ttttgggga  5339
```

<210> 25
<211> 547
<212> PRT
<213> Arabidopsis thaliana

<400> 25

```
Met Ala Ile Leu Asp Ser Ala Gly Val Thr Thr Val Thr Glu Asn Gly
  1               5                  10                  15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg
             20                  25                  30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser
         35                  40                  45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp
     50                  55                  60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp
 65                  70                  75                  80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu
```

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro
100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe
115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile
130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Ser His Ala
145 150 155 160

Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile Ala Val Asn Ser Arg
165 170 175

Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp Leu Ile Arg Thr Asp
180 185 190

Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp Pro Leu Phe Met Cys
195 200 205

Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala Phe Thr Val Glu Lys
210 215 220

Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val Val Ile Phe Leu His
225 230 235 240

Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro Val Tyr Val Thr Leu
245 250 255

Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr Leu Met Leu Leu Thr
260 265 270

Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala His Thr Ser Tyr Asp
275 280 285

Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala Asn Pro Glu Val Ser
290 295 300

Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe Met Val Ala Pro Thr
305 310 315 320

Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala Cys Ile Arg Lys Gly
325 330 335

Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile Phe Thr Gly Phe Met
340 345 350

Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile Val Arg Asn Ser Lys
355 360 365

His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile Glu Arg Val Leu Lys
370 375 380

```
Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys Met Phe Tyr Cys Phe
385                 390                 395                 400

Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu Leu Cys Phe Gly Asp
                405                 410                 415

Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys Ser Val Gly Asp Tyr
            420                 425                 430

Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met Val Arg His Ile
        435                 440                 445

Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys Thr Leu Ala Ile Ile
    450                 455                 460

Ile Ala Phe Leu Val Ser Ala Val Phe His Glu Leu Cys Ile Ala Val
465                 470                 475                 480

Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu Gly Ile Met Phe Gln
                485                 490                 495

Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln Glu Arg Phe Gly Ser
            500                 505                 510

Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe Cys Ile Phe Gly Gln
        515                 520                 525

Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu Met Asn Arg Lys Gly
    530                 535                 540

Ser Met Ser
545
```

Original (for **SUBMISSION**) - printed on 16.12.1999 03:08:17 PM

0-1	**Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared using	PCT-EASY Version 2.90 (updated 15.10.1999)
0-2	**International Application No.**	
0-3	**Applicant's or agent's file reference**	43922-PT

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	page	32
1-2	line	19
1-3	**Identification of Deposit**	
1-3-1	Name of depositary institution	American Type Culture Collection
1-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
1-3-3	Date of deposit	29 November 1999 (29.11.1999)
1-3-4	Accession Number	ATCC PTA-988
1-4	**Additional Indications**	NONE
1-5	**Designated States for Which Indications are Made**	all designated States
1-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	page	32
2-2	line	20
2-3	**Identification of Deposit**	
2-3-1	Name of depositary institution	American Type Culture Collection
2-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
2-3-3	Date of deposit	29 November 1999 (29.11.1999)
2-3-4	Accession Number	ATCC PTA-989
2-4	**Additional Indications**	NONE
2-5	**Designated States for Which Indications are Made**	all designated States
2-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
3	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
3-1	page	32
3-2	line	21

Original (for **SUBMISSION**) - printed on 16.12.1999 03:08:17 PM

3-3	**Identification of Deposit**	
3-3-1	Name of depositary institution	American Type Culture Collection
3-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
3-3-3	Date of deposit	03 December 1999 (03.12.1999)
3-3-4	Accession Number	ATCC
3-4	**Additional Indications**	NONE
3-5	**Designated States for Which Indications are Made**	all designated States
3-6	**Separate Furnishing of Indications**	Accession number of Deposit dated Dec 3, 1999
	These indications will be submitted to the International Bureau later	

FOR RECEIVING OFFICE USE ONLY

0-4	**This form was received with the international application:** (yes or no)	yes
0-4-1	Authorized officer	Sophie Hadeau

FOR INTERNATIONAL BUREAU USE ONLY

0-5	**This form was received by the international Bureau on:**	
0-5-1	Authorized officer	

PCT/CA99/01202

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 32, line 21

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

10801 University Boulevard
Manassas, Virginia 20110-2209, U.S.A.

Date of deposit	Accession Number
December 3, 1999	PTA-1013

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/01202

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/54 C12N15/82 A01H5/00 A01H5/10 A01H3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R61u012 Database Entry Ac005917 Accession number AC005917; 4 November 1998 LIN X. ET AL.:"Arabidopsis thaliana chromosome II section 113 of 255 of the complete sequence" XP002133608 nucleotides 21090-23400 -& LIN X. ET AL. : "Sequence and analysis of chromosome II of Arabidopsis thaliana" NATURE, vol. 402, 1999, pages 761-768, XP000877287 LONDON GB --- -/--	1-4,6-8, 10-13, 19-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2000	14/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Montero Lopez, B

6

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/01202

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CASES S ET AL: "Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 95, no. 22, 27 October 1998 (1998-10-27), pages 13018-13023, XP002122745 ISSN: 0027-8424 cited in the application the whole document	1,2,22
X	VESNA KATAVIC ET AL.: "Alteration of seed fatty acid composition by an ethyl methanesulfonate-induced mutation in Arabidopsis thaliana affecting diacylglycerol acyltransferase activity" PLANT PHYSIOLOGY, vol. 108, 1995, pages 399-409, XP002915657 cited in the application page 399, right-hand column, last paragraph -page 400, left-hand column, paragraph 1 page 402, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 403, right-hand column, last paragraph -left-hand column, paragraph 1 page 405, right-hand column, last paragraph -page 408, left-hand column, paragraph 1	10-18
E	WO 99 67403 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 29 December 1999 (1999-12-29) page 3, line 27 -page 6, line 5 page 10, line 2 - line 25 page 14, line 16 - line 21 page 15, line 24 -page 16, line 28 page 33, line 4 - line 32 page 39, line 28 -page 40, line 11	1-4,6, 10,12, 19-23
E	WO 00 01713 A (CALGENE LLC) 13 January 2000 (2000-01-13) page 4, line 10 - line 17 page 4, line 20 -page 5, line 2 page 11, line 27 -page 24, line 21; examples 11-14,16	1-4,6, 10,12, 19,22,23

-/--

6

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	Empln Database Entry Ath238008 Accession number AJ238008; 18 June 1999 ZOU J. ET AL.:"The Arabidopsis thaliana TAG1 gene encodes for a diacylglycerol acyltransferase" XP002133609 cited in the application the whole document	1,2,22
P,X	ZOU, JITAO ET AL: "The Arabidopsis thaliana TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene" PLANT J. (1999), 19(6), 645-653 , XP002133607 the whole document	1,2,22
P,X	EMBL Database Entry AF155224 Accession number AF155224; 30 June 1999 NYKIFORUK C.L. ET AL.:"Brassica napus putative diacylglycerol cyltransferase (DGAT2) mRNA" XP002133639 cited in the application the whole document & NYKIFORUK C.L. ET AL.: "Isolation and sequence analysis of a novel cDNA encoding a putative diacylglycerol acyltransferase from a microspore-derived cell suspension culture of Brassica napus L. cv Jet Neuf (Accession No. AF155224) (PGR99-123)." PLANT PHYSIOLOGY, vol. 120, no. 4, 1999, pages 1207-1207, LONDON GB	1,2,22
P,X	EMBL Database Entry AF164434 Accession number AF164434; 26 July 1999 NYKIFORUK C.L. ET AL.:"Brassica napus putative diacylglycerol acyltransferase (DGAT1) mRNA" XP002133640 cited in the application the whole document & NYKIFORUK C.L. ET AL.: "Isolation and characterization of a cDNA encoding a second putative diacylglycerol acyltransferase from a microspore-derived cell suspension culture of Brassica napus L. cv Jet Neuf (Accession No. AF164434) (PGR99-158)" PLANT PHYSIOLOGY, vol. 121, no. 3, 1999, pages 1053-1053,	1,2,22

-/--

6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/01202

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	HOBBS D H ET AL: "Cloning of a cDNA encoding diacylglycerol acyltransferase from Arabidopsis thaliana and its functional expression" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 452, no. 3, 11 June 1999 (1999-06-11), pages 145-149, XP002122747 ISSN: 0014-5793 the whole document	1,2,22
P,X	WO 99 63096 A (CALGENE LLC) 9 December 1999 (1999-12-09) page 3, line 24 -page 4, line 17 page 6, line 13 -page 8, line 16 page 12, line 30 -page 20, line 10; examples 1-4,7,8 sequence listing SEQ ID NO:1	1-4,6, 10-13, 19,22,23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 99/01202

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9967403 A	29-12-1999	WO 9967268 A	29-12-1999
WO 0001713 A	13-01-2000	NONE	
WO 9963096 A	09-12-1999	NONE	

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 21 August 2000 (21.08.00)	
International application No. PCT/CA99/01202	**Applicant's or agent's file reference** 43922-PT
International filing date (day/month/year) 16 December 1999 (16.12.99)	**Priority date** (day/month/year) 17 December 1998 (17.12.98)
Applicant ZOU, Jitao et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

14 July 2000 (14.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Nestor Santesso

Telephone No.: (41-22) 338.83.38

CA9901202

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.